

On the Cytology of the Neurons of Cephalopods.

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With Plates 1-6 and 2 Text-figures.

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I. INTRODUCTION.

A REINVESTIGATION of the cytology of the neurons of molluscs seemed to be desirable for at least three different reasons. In the first place their large size makes them suitable for the investigation of those obscure substances in the cell usually classed together as 'Golgi apparatus'. The nerve-cells of *Helix* have been studied from this point of view by several workers, among the more recent being Kunze (1921), Brambell (1923), and Brambell and Gatenby (1923), from whose work it appears that the 'Golgi apparatus' consists of rod- or ring-shaped dictyosomes each enclosing a chromophobe 'archoplasm'. The nerve-cells of Cephalopods have been examined by Garaieff (1909), and Weigl (1910 and 1912), and recently by Parat (1928), who studied the cytoplasmic inclusions of both Gastropods and Cephalopods. He revealed the vacuoles, stainable with neutral red, which he holds to be the living counterpart of the 'Golgi apparatus' which is seen in the fixed cells. Thus the whole question of the nature of these substances is involved, and it seemed worth while to determine whether the view of Parat is really correct. A discussion of the whole subject appears below.

The second aspect for consideration concerns the Nissl substance. This has been intensively studied in vertebrates, but there are only a number of scattered and imperfect observations as to its distribution in invertebrates. This substance is so characteristic of all nerve-cells that it seemed desirable to fill this gap in our knowledge by an accurate study of it in a single group. The Cephalopods lend themselves readily on account of the large size of the cells, and also because with them it is possible to make many of the experiments (section of the axons, stimulation of the nerves, &c.) which have been used in the study of the vertebrate nervous system.

Thirdly, a study of these cells might be expected to throw some light on the architecture of the nervous system itself, which is perhaps more complex in the Cephalopods than in any other invertebrate, although we have very little information as to its finer structure.

The animals studied were *Sepia officinalis* (L.), *Loligo vulgaris* (Lam), *Octopus vulgaris* (Lam), *Octopus macropus* (Risso), and *Eledone moschata* (Leach). The material was collected at Naples during 1929 and 1930 while in occupation of the Oxford Table, and during tenure of the Christopher Welch scholarship and of a Senior Demyship from Magdalen College, Oxford. I should like to thank all the bodies concerned for furnishing these funds, and also Professor R. Dohrn and the staff of the Stazione Zoologica for their help. To Professor E. Sereni, the Director of the Physiological Department of the Station, I am most deeply indebted, not only for indicating the possibilities of study offered by the Cephalopods, but also for providing much of the material and for continual help and advice throughout the work. I have also to thank Professor E. S. Goodrich and Professor R. A. Peters for suggestions and advice and Mr. G. R. de Beer and Mr. R. Palmer for reading the manuscript.

The cells studied were taken mostly from the stellate ganglion, occasionally from the peri-oesophageal ganglia. The structure and functions of the stellate ganglion are not completely known. The cells are very resistant to the usual nerve stains and their finer ramifications have not yet been revealed. From physiological evidence (Bozler, 1927; Hanström, 1928; Sereni, 1929) it appears that the ganglion is a relay in the motor innervation of the muscles of the mantle. Fibres running backwards in the mantle connective end in the ganglion, and the axons of the cells of the latter run out in the stellar nerves to end in the muscles (Text-fig. 1). The ganglion is probably not a reflex centre, and the sensory fibres pass through it without synapse on their way to the peri-oesophageal centres. However, stimulation of the central end of a cut stellar nerve sometimes causes contraction of a distant part of the mantle (Fröhlich, 1910), this is possibly an axon reflex. The arrangement in *Sepia* and *Loligo* differs somewhat from that in the *Octopoda* on account of the presence, in the former, of a fin which is innervated by a separate nerve which does not run into the stellate ganglion.¹

¹ Ten Cate (1929) has recently maintained that the fibres for the innervation of the hind part of the fin of *Sepia* actually pass through the ganglion

The general plan of the stellate ganglion resembles that of most invertebrate ganglia, with several layers of unipolar nerve-cells on the outside and a network of fine fibres (neuropil) at the centre. In order to investigate the ramifications of the cells, the specific neurological methods of Golgi, Bielschowsky, and Cajal were used and modified in various ways, but only the last gave valuable results (an account of the modifications used will be published later). With this method it was seen that the neurons of the stellate ganglion are unipolar; the axons run as single threads about $2-5\mu$ thick as far as the neuropil, where they divide into a number of branches which become lost in the complex network of fibres. It seems that not all the connexions are made in the neuropil, but that some of the finer branches run up between the cells themselves; no terminal apparatus was ever seen and the nature of these endings remains obscure.

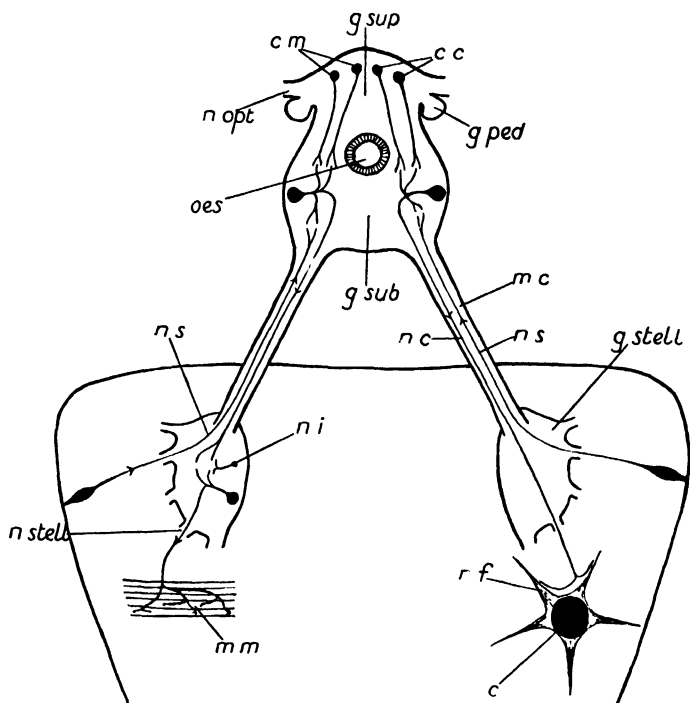
II. OBSERVATIONS ON THE LIVING CELLS.

1. Unstained.

Thick hand sections of ganglia of *Octopus*, *Eledone*, and *Sepia* were studied, in sea-water, with a 2 mm. oil-immersion objective. The nuclei were clearly outlined, and within each two or three colourless nucleoli and other smaller granules. Around the nucleus could be seen a mass of colourless spheres of various sizes: as will be shown below these are really vacuoles (vesicles). No differentiated structures could be seen in the outer part of the cytoplasm, which appeared to be very finely granular. There was no visible Brownian movement in any part of the cells.

and thence out in the stellar nerves. I have never found this to be the case. After all the stellar nerves had been cut, leaving only the n. pinnae in connexion with the mantle, then electrical stimulation of the mantle connective near to the 'brain' gave movements of all parts of the fin. Controls for escape of current were made, and the fin was cut into a number of sections to ensure that the movements of the hind part were not simply passive and due to the movements more anteriorly. Conversely, after the n. pinnae alone had been cut, stimulation of the mantle connective caused no movements of the fin. It seems, therefore, that all the fibres run to the fin in the separate n. pinnae and none in the stellar nerves.

When examined with polarized light the fine connective tissue-fibres between the cells and in the neuropil were found to be strongly anisotropic and shone very clearly. The nerve-fibres themselves did not seem to be anisotropic either in the ganglia or nerve-trunks. Within the cells themselves the only



TEXT-FIG. 1.

Diagram of the connexions of the stellate ganglion of Octopoda.

The fibres for the mantle muscles are shown on the left, those for the chromatophores on the right. (Compiled with the assistance of Professor Sereni.) *c*, chromatophore; *cc*, higher motor and inhibitory centres for the control of the chromatophores; *cm*, higher centres for the control of the mantle muscles; *g ped*, ganglion pedunculatum; *g stell*, ganglion stellatum; *g sub*, suboesophageal ganglion; *g sup*, supra-oesophageal ganglion; *mc*, mantle connective; *mm*, mantle muscles; *nc*, nerve-fibre to chromatophores; *ni*, intercalary neuron; *n opt*, optic nerve; *ns*, sensory fibres; *n stell*, stellar nerve; *oes*, oesophagus; *rf*, radial muscle-fibre of chromatophore.

parts to show anisotropy were the vacuoles around the nucleus. These showed up quite clearly in some cells, but the anisotropy was never so marked as that of the connective tissue-fibres.

2. Staining with Neutral Red.

Solutions were made up by diluting a 1 per cent. solution of neutral red (made up in distilled water) with sea-water. Concentrations of from 1/20,000 to 1/1,000,000 were used. When a hand section of a stellate ganglion was placed in a dish containing neutral-red solution the result was invariably a selective stain of the spheres which could be seen in the unstained cells around the nucleus (figs. 1 and 2, Pl. 1). With the more concentrated solutions the staining took place very rapidly (five minutes or less), with weaker solutions up to half an hour was necessary. In all cases it was the larger bodies which stained first and most intensely, and if weak solutions were used for only a short time then the smaller remained unstained (fig. 2, Pl. 1). The mass of red bodies was often restricted to the perinuclear region, but in many of the larger cells (especially of *Sepia*) it extended out as arms along certain radii almost to the surface of the cell. In most cases the larger vacuoles lay near the outer edges of the mass, and there were often several of them so close to one another that the boundaries could hardly be distinguished. Often there was one group of large vacuoles on the side of the cell towards the axon and another at the opposite pole, but some were also seen to lie laterally. The stain was very strictly localized in the vacuoles, the rest of the cytoplasm and the nucleus remaining unstained. It is necessary to stress the fact that these bodies which stained with neutral red were indubitably the same as the colourless spheres seen in unstained pieces and were not neoformations such as were seen after neutral-red staining by Chlopin (1927) and Ludford (1930). I have no doubt that they exist in the same form in the normal cell, during life. It is not possible to prove this, since the cells cannot be observed without removal from the body, but the technique of cutting thick hand sections and examining in an isotonic solution is the best possible under the circumstances. The vacuoles appear identical whether the cells are examined in

this way one minute or several hours after removal from the body, and perfectly corresponding figures are obtained by fixing whole ganglia or thick sections in a variety of ways (see below). It is certain that such stable and constant structures pre-exist in the living cell and are not the result simply of removal from the body and transference to an isotonic solution.

In a preparation protected against evaporation the stain remained unchanged for 3-4 hours, but ultimately the cells died and the colour became diffused throughout the nucleus and cytoplasm. In ganglia removed from the body and kept in running sea-water, it was found that even after 24 hours there were often bodies present which stained with neutral red; these were much smaller than the normal vacuoles, however, and gave the appearance of a powder round the nucleus. The capacity to stain with neutral red disappears before disintegration of the vacuoles, so that ganglia could be obtained in which the cells contained what appeared to be perfectly normal vacuoles, but neutral red gave only a diffuse stain of the nucleus and cytoplasm. This would seem to show that the staining of the vacuoles depends on some chemical reaction and not on absorption or concentration of the stain in a particular phase of the system.

Ganglia (*Octopus vulgaris*) were cut out of the body and kept for $6\frac{1}{2}$ hours at tank temperature, one in 50 c.c. of sea-water and the other in 50 c.c. of a N/10,000 solution of KCN in sea-water. Sections of these ganglia examined unstained revealed no differences, both large and small vacuoles being visible in all cells. However, in the cells treated with KCN the vacuoles stained only very faintly with neutral red, whereas in the control cells they stained readily.

In another experiment ganglia (*Octopus vulgaris*) were cut out and kept for 10 hours at tank temperature, one in 50 c.c. of sea-water and the other in sea-water, previously warmed for 10 minutes to $70^{\circ}\text{C}.$, through which nitrogen had then been bubbled for $\frac{1}{2}$ hour to expel all the oxygen (and CO_2 , so that after this treatment the pH rose a little). At the end of this time the neutral-red vacuoles appeared to be perfectly similar in the two ganglia. It seems, then, that the vacuoles

are not entirely dependent on aerobic respiration for their maintenance.

Sections of ganglia were placed in solutions of neutral red made up in sea-water to which had been added $3\frac{1}{2}$ per cent. and 7 per cent. of sodium chloride, so that their osmotic pressures were roughly two and three times that of normal sea-water. Under these conditions it was seen that the liquid which was stained with neutral red no longer occupied the whole volume of the vacuoles, but that it had shrunk away to one side, leaving a space which was only faintly tinged with red. When $3\frac{1}{2}$ per cent. of salt had been added, the red liquid occupied about half of the vacuoles (fig. 4, Pl. 1), while with 7 per cent. it shrank to small droplets at the edges (fig. 5, Pl. 1); the surrounding cytoplasm seemed to be slightly tinged with red. Pieces treated in this way for up to 1 hour were then replaced in ordinary sea-water, and all the vacuoles refilled and appeared uniformly coloured with red. They were then again transferred to hypertonic sea-water and there was the same shrinkage of the contained liquid, the outlines of the vacuoles remaining perfectly clear.

These experiments were repeated several times, and they indicate very clearly that the bodies in question are true vacuoles (vesicles) with an outer wall and containing a watery solution. The most probable explanation of the phenomena observed would seem to be that the wall is permeable to water and to neutral red, but impermeable to inorganic ions; hence, when surrounded by a hypertonic salt solution water passed out, leaving the vacuole only partly filled. The quantity which passed out was naturally greater when the osmotic pressure was increased three times than when it was doubled. It is remarkable that the walls of the vacuoles are strong enough to resist the tension which must be set up when liquid passes out in this way: it would be expected that they would collapse. It is difficult to understand what it can be that occupies the part of the vesicle empty of red fluid; possibly part of the lipid (?) constituents of the wall are drawn off. That the vacuoles are indeed very stable structures is further shown by the fact that if the cells are compressed under the cover-slip they disintegrate, and the

stained vacuoles can be seen to emerge and maintain their identity outside the cell.

There is an important distinction between such vesicles and droplets. In the case of the latter there may be an interphase membrane where the substance composing the droplet meets the surrounding medium, but there is no formed membrane differing in constitution from the liquids on both sides of it. Parat has constantly assumed that the bodies which stain with neutral red are vacuoles (by which presumably he means vesicles), but has not brought any evidence that they have an outer semi-permeable membrane, and are not simply homogeneous droplets, lying free in the cytoplasm.

Analogous effects were seen when freezing-sections of fresh ganglia were stained with neutral red. The stain was taken up very slowly, and in many of the vacuoles only a part of the contents stained, although the outline of the wall of the vacuole could clearly be seen (fig. 6, Pl. 1).

When sections were placed in sea-water diluted with 50 per cent. of distilled water, the vacuoles remained intact, the only abnormality being that they seemed to be unusually sharply marked off from the ground cytoplasm. If sections, previously stained with neutral red, were transferred to distilled water the vacuoles immediately broke down and disappeared, the stain becoming diffused throughout the cytoplasm. The vacuoles also disappeared when the pieces were placed in 5 per cent. formol, in Da Fano's or Champy's fixatives or in 2 per cent. osmium tetroxide, if these were dissolved in distilled water; whereas they remained intact in all these fixatives if made up with sea-water.

In pieces fixed in 2 per cent. osmium tetroxide (in distilled water), 1 part, and sea-water, 3 parts, the vacuoles gradually blackened. After 10-15 minutes the larger ones could be seen as darker brown spheres against the lighter brown background of the cytoplasm. They appeared to be quite homogeneous, and there was no indication of an outer rim staining black before the rest of the vacuole, such as was seen by Sharga (1927) and Nath (1930) in the eggs of the earthworm *Pheretima*, and by Covell and Scott (1928) in vertebrate neurons, and which

has been supposed to represent the Golgi substance (as will be shown below the latter is indeed found near the vacuoles, but in the form of granules and not of a continuous ring). After longer immersions in osmium tetroxide the cells became so dark that their internal structure could only be made out with difficulty. After immersion for 40 minutes the vacuoles could be discerned only in some of the smaller cells; they appeared exactly as before, though somewhat darker.

3. Staining with Methylene Blue.

Sections of ganglia were placed in a dish in a solution of methylene blue ('Ehrlich', Grüber) 1/10,000, dissolved in seawater, and after times varying from a few minutes to $\frac{3}{4}$ hour they were removed to a drop of the same solution on a slide, and, when blue, covered and examined. Other pieces so treated were fixed either in 8 per cent. ammonium molybdate (dissolved in distilled water) for about 12 hours or else first in saturated picric acid (in distilled water) for 1 hour and then in ammonium molybdate. After washing, these pieces were dehydrated, cleared in xylol, and mounted in Canada balsam.

In many cases methylene blue stained all the vacuoles round the nucleus. In other cases the stain was taken up not only by the vacuoles, but also by granules placed around them (fig. 13, Pl. 2). These granules correspond in position to the Golgi bodies which are seen after staining with silver or osmium salts, and it is tempting to regard them as such, but further observations must be made before this can be accepted as certain.

4. Staining with Janus Green.

Many unsuccessful attempts were made to stain the chondriosomes with specimens of janus green made by Grüber and Poulenc. Janusgrün B of the I/G Farbenindustrie (Hoechst) was only obtained when it was too late to make more than a few preliminary observations. It was used in concentrations of 1/5,000 to 1/30,000, both on unstained sections and after staining with neutral red. The preparations were far from clear, since the stain killed the cells and the pieces became opaque and difficult to study. In some cases whole small vacuoles were seen

stained green, but in other cases either a part of the surface (or contents) was stained or a number of granules were seen at the surface of the vacuoles, occasionally even of the larger ones (fig. 9, Pl. 2). The smaller vacuoles appeared to lose their red stain when they were stained also with janus green, but many of the larger ones could be seen to retain it. The interpretation of these appearances is difficult. The fact that some substance round the outside of the larger vacuoles stains with janus green, if confirmed, would support the hypothesis of Parat that the argentophile, osmiophile granules (Golgi bodies), which are also to be found in this region, are of similar nature to the chondriosomes. The green stain in connexion with the smaller droplets seems to correspond to the picture of the mitochondria seen after fixation (p. 22). Parat (1928) reports that with janus green 'le chondriome est représenté par des fins chondriocentes repartis dans la cellule de façon assez uniforme'.

5. 'Oxidase' Reactions.

(a) Indophenol Blue.

Solutions of α -naphthol and dimethyl-paraphenylenediamin were made up to strengths of 0.1 per cent. in sea-water. They were used fresh or at latest on the day after dissolving. After sections of the ganglia had been cut, the two solutions were filtered and mixed in equal parts, giving a colourless solution, in which the sections were placed and removed at intervals for examination. After about 10 minutes the liquid became distinctly blue and the cells took on a diffuse pale-blue tinge, the larger vacuoles standing out on account of a slightly darker stain. After 20-30 minutes a number of intensely blue granules appeared in the cells, and of these by far the greater part, including the largest ones, were situated at the surface of the vacuoles, giving them a beaded appearance (figs. 11 and 12, Pl. 2). A certain number of granules were scattered through the cytoplasm (mostly near the nucleus), and it could not be determined whether these were accompanied by small vacuoles or not.

If the piece was left under a cover-slip the blue stain faded, until after about an hour no granules could be seen.

If the vacuoles were stained with neutral red before placing in the reaction mixture, it could be seen that the blue granules formed round red vacuoles, but the red colour rapidly disappeared and was replaced by blue.

These appearances were regularly seen in eight experiments. In three others there were seen in addition to the small granules described above, a number of large, deep blue globules, each surrounded by a more lightly stained area (vacuole?) and scattered throughout the whole cytoplasm. This stain persisted under a cover-slip without fading, and it is probable that the cells in question were injured or dead.

In one experiment KCN was added to the reagents to give a concentration of N/2,000. The liquid gradually turned blue and had the same tinge as the control, but the cells took only a diffuse blue stain and no blue granules were observed.

(b) Paraphenylenediamin.

Further experiments were made, using paraphenylenediamin (0.1 per cent.) dissolved in sea-water. This gave a clear solution which gradually acquired a reddish-black tinge. In pieces (sometimes previously stained with neutral red) placed in such a solution black rims appeared, after 25–45 minutes, round many of the vacuoles (figs. 7 and 8, Pl. 1). These rims were quite distinct, especially if the vacuoles had first been stained with neutral red and were not simply an optical effect. At about the same time black deposits began to appear in the form of granules and rods, either free in the cytoplasm near the outer surface of, or even actually inside the vacuoles (in which case they were often of a characteristic dumb-bell shape). If the piece was left under the cover-slip the black rims faded away in the course of about half an hour, whereas the black granules and 'dumb-bells' remained.

After a longer stay (1–2 hours) in the paraphenylenediamin solution a mass of black granules, rods, and crystals was seen in the perinuclear space; the vacuoles could no longer be made out. In every cell there was such a mass in the space occupied by the

vacuoles, but in no case were more than one or two crystals seen in the rest of the cytoplasm or the nucleus (fig. 10, Pl. 2).

(c) Discussion.

These reactions with 'indophenol' reagent and p-phenylenediamin have been objected to as indicators of localized oxidation (or oxidases) in the cell on three grounds: (a) that the coloration is due to 'basic staining' of preformed granules (Hollande, 1924); (b) that the formation of the coloured substance is the effect of a surface or interphase and does not necessarily indicate the presence of oxidizing substances at the points coloured (Lillie, 1913; Menten, 1919); (c) that in any case the reactions give no possibility of determining the oxidation-reduction potential in absolute terms (Needham and Needham, 1925, 1926, 1927; Clark, Cohen, and Gibbs, 1926).

Objection (a) cannot be altogether excluded, and it is not necessarily to be maintained that the granules seen with the indophenol-blue reaction represent preformed oxidase granula; but the coloration was so intense that it is highly probable that there was actual synthesis of indophenol blue in those regions (whereas the vacuoles, which do certainly stain by 'basic staining' or concentration of the dye, were much paler). However, the bodies seen after the use of p-phenylenediamin were undoubtedly neo-formations, crystals deposited in a certain region. This also disposes of the second objection since the crystals were formed between the vacuoles as well as on their surfaces, and indeed often actually inside them, so that they cannot have been the result only of surface effects. A more legitimate objection would be to suppose that the reagent was concentrated and restricted by the activity of each cell to its central region. Such an explanation of the localized coloration does not, however, cover the case of the indophenol-blue reaction, where there was a faint blue stain throughout the cells, showing that the reagent was present all over, though actively oxidized only in a certain region. Local concentration cannot be excluded as the explanation of the distribution of the stain with p-phenylenediamin, but it seems unlikely in view of the fact that it was the central part of the cells which became coloured; if the

oxidation potential of the outer region was high it is improbable that the reagent would pass through it without leaving considerable traces. As regards the third objection: certainly it is desirable, wherever possible, to determine the oxidation-reduction potential of any living system with reference to an agreed scale. This cannot be done with the above indicators, and it is unfortunate that those studied by Clark were not available. However, what the experiments do seem to indicate quite definitely is that the oxidation-reduction potential is not uniform throughout the cell, but that oxidation goes on more readily in a certain region. This is in agreement with the general ideas of Parat (1928) on this subject. The Needhams and Rapkine and Wurmser (1926), on the other hand, seem to regard the entire cell, including the nucleus, as a homogeneous oxidation-reduction system.

It is of interest that Keilin (1929) considers that the p-phenylenediamin- (indophenol-) oxidase which mediates the oxidation of these reagents by yeast, is one of those responsible for oxidation in the living cells, and that it is in fact identical with Warburg's 'respiratory ferment'.

III. OBSERVATIONS ON FIXED MATERIAL.

1. Methods.

In order to secure proper penetration of the fixative it was necessary to use thick hand sections of the ganglia, since there is an outer sheath of connective tissue which hinders penetration, especially of osmium tetroxide. It was found to be of the greatest importance to use only fixatives made up in sea-water, since hypotonic solutions caused bursting and disintegration of the vacuoles (p. 9).

Kolatschev's method was found to be one of the best for demonstrating the Golgi bodies and vacuoles. Thick hand sections of ganglia were fixed in

1 per cent. chromic acid dissolved in sea-water .	7 parts
3 per cent. potassium bichromate dissolved in sea-water	7 „
2 per cent. osmium tetroxide dissolved in distilled water	4 „

After fixation for 24 hours the pieces were washed and stained

with 1 per cent. osmium tetroxide at 35° C. for 5-6 days and then embedded in wax and sectioned (3-6 μ). Sections were mounted directly in balsam or cedar-wood oil, or else partly bleached with potassium permanganate and then counter-stained with acid fuchsin.

Cajal's and Da Fano's methods were found to be more convenient than Kolatschev for routine use. They were modified as follows:

Uranium or cobalt nitrate	1 gm.
Neutral formol	15 c.c.
Sea-water	85 c.c.

This mixture was used for about an hour and then washed out by means of about five changes of a similar solution made up with distilled water (this is necessary to avoid precipitates with the silver nitrate). The duration of fixation is very important; if prolonged for more than about 10 hours at room temperature (often 25° C. at Naples) the stain was found to be imperfect. The most constant results were obtained by cooling the fixative to 5° C. and using for 3-5 hours. After fixation the pieces were washed quickly in distilled water and left for 24-48 hours in 0.75 per cent. silver nitrate and then reduced, embedded, and sectioned as usual. Some sections were mounted direct and others toned and counterstained with Ehrlich's haematoxylin or acid fuchsin. Cobalt nitrate gave more constant results than uranium nitrate, although good results were obtained from both.

For fixing the chondriosomes Regaud's and Champy's fluids were used, made up, of course, in sea-water. The sections were stained with iron haematoxylin (Regaud) or with Altmann's acid fuchsin method, sometimes with Kull's modifications.

Other techniques employed were those of Holmgren (for his 'trophospongium'), Ciaccio (for lipins), and Lorraine-Smith (for fats); these were not modified, except for the use of sea-water in the fixative.

For study of the Nissl substance fixatives containing alcohol were used. 95 per cent. alcohol used alone was found to shrink the cells considerably, and this was partly avoided by the addition of nitric acid; if too much was added, however, the

subsequent stain was very faint. 3.5 per cent. (by volume of acid sp. gr. 1.20) was found to give the best results. Carnoy's fluid preserved the shape of the cells and the nucleus better than alcohol, but after its use the Nissl substance had a foamy appearance; it was found to be essential for fixation of the large cells of *Sepia* and *Loligo*. The pieces were embedded in paraffin wax and sections cut $5-8\mu$ thick.

For staining the Nissl substance toluidin blue was used (0.1-1 per cent.); good results were obtained both by heating the slide and stain over a flame for a few minutes and by leaving the slides for some hours in the stain at 35°C . After swilling in tap-water the slides were transferred to 8 per cent. ammonium molybdate for 10 minutes to fix the stain, washed for a few minutes in tap and distilled water, dehydrated in 95 per cent. and absolute alcohol, and mounted in Canada balsam. The sections were sufficiently differentiated in 95 per cent. alcohol, in which the stain readily dissolves.

2. General Observations.

The nerve-cells of the stellate ganglion are of various sizes, the largest being on the outside and the smallest next to the neuropil. In *Sepia* and *Loligo* many of the cells are very large (up to 120μ long and nearly as broad). In *Loligo* the small cells are mostly segregated in a separate lobe of the ganglion. In *Octopus* and *Eledone* the cells are smaller (up to 50μ long) and more numerous.

Each cell is surrounded on all sides by a sheath of connective tissue (possibly also by ectodermal neuroglia cells), and as is well known (Garaieff, 1909; Jakubski, 1915) this is continued into canals in the cytoplasm of the nerve-cells themselves. This presumably constitutes a device for increasing the absorptive surface of the cell, which tends to become less, relative to the volume, as cells become larger. The fibres accordingly penetrate much more deeply into the larger cells of *Sepia* and *Loligo* than into those of *Octopoda*, where they are restricted to the outer layers. Schreiber (1930)¹ noticed eosinophil granules

¹ I have to thank Dr. Schreiber for his kind permission to read his paper before its publication.

round the nuclei in the canals and hence supposed the cells to be amoebocytes. Possibly a few of the nuclei are of this nature, but certainly the majority belong to the connective tissue (see figures of Jakubski (1915) and Weigl (1910), and fig. 16, Pl. 3, of this paper).

The nuclei of the neurons, as seen after fixation in alcohol or Carnoy's fluid and staining with toluidin blue, contain a single karyosome, lying usually, but not always, on the side of the nucleus nearest to the axon. In pieces fixed with Champy's fluid and stained by Kull's method there can be seen in addition, in many of the nuclei, one or two oxyphil plasmosomes, stained red with the acid fuchsin (figs. 14, Pl. 2, 24, Pl. 3). These bodies are apparently dissolved away after fixation in alcohol or Carnoy's fluid, but they appear in cells fixed with formalin. Schreiber (1930) believes that they are to be interpreted as granules of pigment. The nature of these bodies, as of oxyphil nucleoli in other cells, remains obscure.

3. Golgi Bodies, Vacuoles, and Chondriosomes.

(a) Statement of Problem.

The complex of cell-constituents usually classed together as 'Golgi apparatus', 'Golgi element', or 'Golgi region' has been much studied in recent years, but no general agreement has been reached as to its nature or functions. Summaries of this work will be found in the papers of Bowen (1926), Jacob (1926), Nath (1927), Hirschler (1927), Parat (1928), Hertwig (1929), and Gatenby (1930). In 1924 Parat and Painlevé drew attention to the vacuoles present in many cells which are distinguished by their affinity for neutral red. They suggested that the Golgi bodies seen after fixation and staining with silver or osmium are artifacts due to the poor preservation of the vacuoles. In his further elaboration of the hypothesis Parat has suggested that the chondriosomes are of two sorts, ordinary and modified, the latter ('chondriome actif') occurring in the region of the vacuoles and being indeed the 'dictyosomes' (in the cases where

the latter are actually present and not simply artifacts due to distortion of the vacuoles in fixation).

Several authors have accepted this hypothesis in whole or in part: Chlopin (1925 and 1927), Joyet-Lavergne (1926), Poisson (1927) (in part), Feyel (1928), and especially Zweibaum and Elkner (1927 and 1930), Covell and Scott (1928), and Cowdry and Scott (1928). On the other hand, it has been opposed on the grounds (a) that some at least of the vacuoles are products of the action of neutral red on the cells and do not exist at all under normal conditions (Chlopin, 1927; Ludford, 1930; Weiner, 1930); or (b) that, besides the vacuoles, there are other distinct osmiophile and argentophile bodies present in the cell.¹ These two constituents have never been made out together in living cells, except possibly in gametocytes, where the homologies of the various bodies are doubtful (Karpova, 1925; Voinov, 1927; Gatenby, 1929; Bhattacharya and Das, 1929; Nath, 1930; Hirschler and Hirschlerowa, 1930). Neither have they frequently been seen together in fixed cells; Beams (1930) has recently claimed that the osmiophile bodies and vacuoles are separate in the acinar cells of the pancreas, but his observations do not entirely agree with those of Ludford (1930). Tretjakoff (1927) apparently saw both constituents in developing tendons of Amphibia, and Grabowska (1930) claims to have demonstrated them in the cells of the green gland of the crayfish *Astacus (Potamobius)*. Rumjantzew (1928) seems to have distinguished osmiophile substance from neutral-red granules in cultures of mesenchymatous tissues. There are also several

¹ This introduces considerable confusion into the nomenclature. Parat would presumably call all such bodies modified chondriosomes; this seems premature in view of our ignorance of their relation to the ordinary chondriosomes (see below). 'Golgi apparatus' is used by some writers to denote the whole complex, including the vacuoles. Gatenby (1930) has made a good case for excluding the vacuoles from the term, and he restricts it to that constituent which he believes to consist of two parts an osmiophile dictyosome and an osmiophobe 'archoplasm'. In the neurons of Cephalopods no 'archoplasm' has been detected, there are vacuoles and osmiophile granules only; it is proposed to restrict the term Golgi bodies to the latter. This leaves us without a term for the whole complex, but this is not a necessity urgent enough to justify the introduction of a fresh name into an already confused literature.

cases in which trypan blue is known to accumulate in vacuoles in the region where the 'Golgi apparatus' can be demonstrated (Nassonov, 1926; Ludford, 1929), but probably these are segregation droplets not related to the neutral-red vacuoles (v. Möllendorff, 1920; see, however, Ludford, 1930; Nassonov, 1930).

It seems then that only Beams and Grabowska have so far succeeded in clearly distinguishing osmiophile (Golgi) bodies and vacuoles in somatic cells. However, it has long been known that the 'Golgi apparatus' comprised two parts, a chromophobe (archoplasm, idiosome, T-substanz of Kopsch, Apparatinhalt of Hirschler) and a chromophile (dictyosome, O-substanz, Apparathülle), and it seems possible that these represent the vacuoles and osmiophile (Golgi) bodies respectively. Gatenby, however, regards the archoplasm as a separate cell-constituent related to the osmiophile bodies and not identical with the vacuoles. The canals of Holmgren (trophospongium) are probably the same as the chromophobic part of the complex (except where they are ingrowths of connective tissue from outside the cells), though Penfield (1922) maintains that they also are a distinct cell-constituent (see below, p. 34).

There remain for consideration certain workers who do not believe in the existence either of vacuoles or Golgi bodies as separate, formed substances in the living cell. Ciaccio (1927) believes that the bodies seen after both intra-vitam staining and fixation are separation products from the lipo-proteins ('ergoplast') which are concentrated in this part of the cell. Walker and Allen (1927) have shown that it is possible to produce artifacts similar to the classical 'Golgi apparatus' by the action of the usual fixatives on mixtures containing lipins.

The problems of the cytoplasmic inclusions of any type of cell may be put in the form of five questions:

1. What is the nature of the bodies which stain with neutral red; do they exist in living, unstained cells; are they droplets or true vesicles?

2. Have they an outer limiting wall, and if so does this stain with osmium and silver and is it the counterpart of the classical 'Golgi apparatus'?

3. Alternatively are there separate osmiophile and argento-phile aggregations (Golgi bodies) present *in vivo* and not constituting the limiting membrane of the vacuoles? If present, what is their relation to the vacuoles?

4. What are the relations of the vacuoles and of the Golgi bodies (if the latter exist) to the chondriosomes?

5. Are there in the cytoplasm any other formed substances (archoplasm or trophospongium) different from all the above?

These are questions which we can attempt to answer, for each single type of cell, with present-day technique. Since the methods used are empirical and their action is so little understood¹ that they tell us little or nothing of the chemical and physical constitutions of the bodies which they reveal, it is impossible to discover the nature of these substances and hence dangerous to draw homologies between different types of cell.

(b) Observations with Osmium and Silver Methods.

The most complete picture of the complex of cytoplasmic inclusions was obtained with the Kolatschev method when the sections were counterstained with acid fuchsin. In such preparations it can be very clearly seen that there is present in these cells a separate Golgi substance (in the narrow sense, see footnote, p. 18) which is distinct from the vacuoles. The latter are perfectly preserved and stained red: around them can be seen a number of small granules, the Golgi bodies, stained intensely black by the osmium tetroxide. The rest of the cytoplasm is a homogeneous grey, and the nucleus, karyosome, and plasmosome are faintly outlined (figs. 2, 14, 18–21, Pl. 1, 2 & 3). The majority of these black granules can be seen to lie close to the surface of the vacuoles, either singly or several aggregated together into larger lumps. A few granules seem to lie alone in the cytoplasm, but probably these are in fact accompanied by smaller vacuoles, not properly preserved or stained. On the other hand, there are always a number of vacuoles which are not accompanied by any granules. It also seems quite clear that

¹ For instance, Rumjantzew (1928) and Zweibaum and Elkner (1930) have pointed out that several different cell-structures may stain with osmium in one single type of cell.

the granules, whatever their true nature, are not products of bad fixation of the outer layers of the vacuoles, as maintained by Parat (1928). The outlines of the vacuoles can be seen to be perfectly distinct and sharp, and they seem to be very well fixed. It is important to observe that the black-staining bodies do not constitute the outer wall or cortex of the vacuoles. The latter have a limiting membrane of their own and the granules lie outside this.

Similar pictures were obtained by the use of Cajal and Da Fano techniques; with these the Golgi bodies proper and the vacuoles were both stained, but with varying intensity, depending, apparently, on the duration of fixation. Usually (after a short fixation, especially if the fixative was cooled) the vacuoles were only slightly stained (yellow in untuned preparations) and the Golgi bodies could be seen as black granules around their outer surfaces (figs. 15, 27-9, Pl. 1 & 4): toning of such preparations often removed all stain from the vacuoles, which then became slightly tinged with red after staining with acid fuchsin. After lengthy fixation, and particularly with uranium nitrate, the vacuoles, especially the larger ones, stained more intensely and the Golgi bodies little if at all, giving appearances such as those seen in figs. 22 and 23, Pl. 3, in which several large vacuoles can be seen close together, in a line. It is easy to understand how such bodies, especially in small cells, would have the appearance of rods, or meshes of network (see fig. 33, Pl. 4), and this is the probable explanation of the form of the classical 'apparato reticolare interno', see Covell and Scott (1928). If the pieces were left for too long in silver nitrate, both Golgi bodies and vacuoles were stained a deep black and could only be distinguished with difficulty. In general these methods give a more complete but less diagrammatic view of the Golgi complex than does Kolatschev's method. Apparently all the vacuoles stain slightly in an optimum silver preparation, and it seems probable that all the darker granules (Golgi bodies) are accompanied by vacuoles.

Parat (1928) stated that he found silver methods preferable to osmium methods for staining the Golgi elements of the nerve-cells of Cephalopods, and that thick sections showed more

than thin ones. This is partly true: thick sections are necessary in order to observe the general topography of the vacuoles and especially the relations of the larger ones. However, for a study of the Golgi bodies themselves and their relations to the vacuoles it is essential that the sections be as thin as possible, and it was probably due to neglect of such study that Parat failed to distinguish the granules from the vacuoles. He observed (and figured) in his thick sections a mass of darkly stained bodies around the nucleus, and concluded that this was entirely composed of the stained vacuoles, which intra-vitam observations had shown to lie in the same region.

(c) Observations with Methods for Fats and Lipins.

Sections of ganglia were treated according to Ciaccio's directions for the fixation of lipins, the fixative being made up in sea-water. Paraffin sections were stained with Sudan III or Nile-blue sulphate and mounted in Apathy's syrup or glycerine. After such treatment the larger vacuoles in the cells were always distinctly stained and stood out well against the unstained background of the rest of the cell. Similarly after fixation in formalin and staining of frozen sections with Nile-blue sulphate (Lorrain-Smith), the larger vacuoles were stained faint blue. If these methods are really specific they indicate that at least the larger vacuoles are composed in part of lipins or fats. This is the conclusion reached by the majority of workers, though denied by Parat. Probably it is the walls of the vacuoles which are composed of fat or lipin, separating watery phases inside and outside.

(d) Chondriosomes.

After fixation in Champy's fluid and staining with acid fuchsin by Altmann's method, both vacuoles and chondriosomes were stained red. The chondriosomes consist of rings or rods scattered through the whole cytoplasm, but apparently more densely aggregated in the perinuclear area (fig. 26, Pl. 3). In two of the preparations in which Kull's modification was used the vacuoles were stained red and the chondriosomes

appeared as minute vesicles, each with two or three spots at the periphery stained deep purple. This appearance corresponds with the pictures obtained after supra-vital staining with janus green. On repetition this result could not again be obtained, both chondriosomes and vacuoles stained red.

Sections stained with iron haematoxylin after fixation in Champy's fluid showed the vacuoles, and sometimes also the chondriosomes, stained black. The vacuoles were beautifully preserved and their outlines appeared to be quite smooth (figs. 24 and 25, Pl. 3), no trace of the Golgi bodies appearing. After fixation in Regaud's fluid and staining with iron haematoxylin both vacuoles and chondriosomes were seen. The latter usually appeared as short rods or rings (as after fixation in Champy), but in some cells the cytoplasm was seen to be full of considerably larger rods, which were often aggregated into quite long chains. These cells were mostly considerably distorted, and it seems probably that such appearances were the result of bad fixation. In some of the cells there was a very large, darkly stained mass within the area occupied by the vacuoles (fig. 17, Pl. 3). This was probably also an artifact.

(e) Fixation with Trichloroacetic Acid.

Sections of ganglia were fixed by Holmgren's method, embedded in paraffin, sectioned and stained with acid fuchsin and iron haematoxylin, which technique should reveal the trophospongium. Actually the nuclei and Nissl substance were seen to be well fixed and stained, while the perinuclear area was partly unstained but contained some dark bodies which could be identified as the remains of vacuoles.

(f) Discussion.

We are now in a position to answer the questions formulated on p. 19.

1. In the neurons of Cephalopods the bodies which stain with neutral red can be seen in the unstained cells; they are true vacuoles (vesicles) having an outer wall which is probably of fatty or lipid nature and containing a watery fluid which combines with neutral red.

2. The vacuoles (or at least their outer membrane) will stain with silver and to some extent with osmium (both when directly immersed in osmium tetroxide and after fixation in Champy's fluid and subsequent osmification), but they do not completely represent the classical 'Golgi apparatus'.

3. There are a number of osmiophile, argentophile granules which lie near to the vacuoles but do not form their outer membrane. These bodies cannot be seen in the living cells (unless they are the counterpart of certain granules which stain with methylene blue), neither do they appear after fixation in Champy's fluid and staining with iron haematoxylin. It cannot, therefore, be certain that they exist in the living cell in the form seen in fixed preparations; there must, however, be some differentiated substance in this region, which gives rise to the granules after treatment with osmium or silver salts.

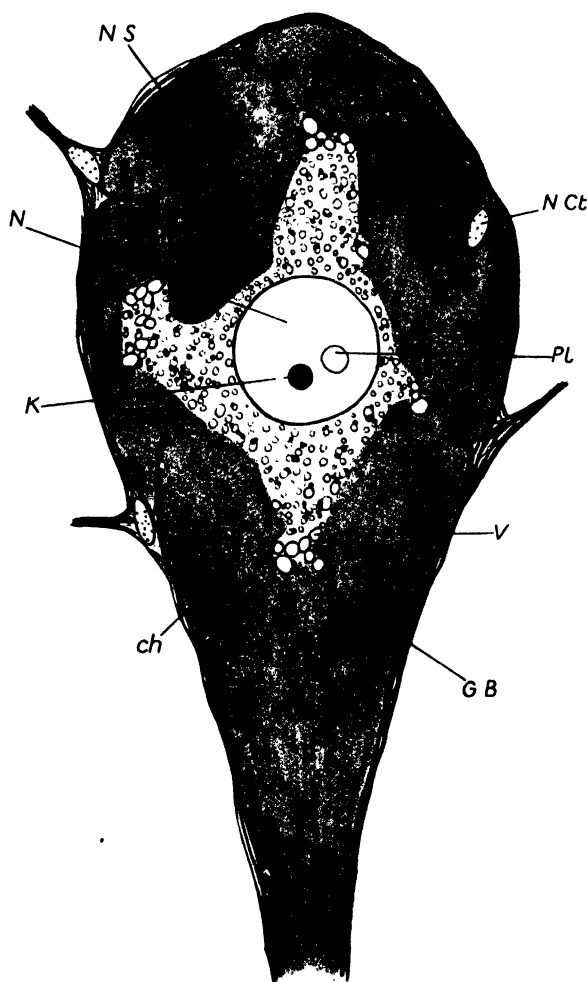
4. The chondriosomes are much smaller than the Golgi bodies and are scattered throughout the cell: they do not stain with osmium or silver, and their relationship to the other inclusions remains obscure, except that there is some evidence (derived from staining with janus green) that they consist of a substance similar to the Golgi bodies.

5. No differentiated substances (archoplasm, trophospongium, &c.) other than the Golgi bodies, chondriosomes, and Nissl substance were recognized in the cytoplasm.

Parat (1928) is thus right in stating that the vacuoles sometimes stain with silver and osmium, but wrong in denying the presence of other distinct osmiophile, argentophile bodies in this region. There is undoubtedly some aggregation around many of the vacuoles, of a substance not present elsewhere in the cytoplasm. Its nature remains unknown, though it is possible that it resembles the substance of the chondriosomes. It is best, therefore, to retain for it the separate term Golgi substance. The relations of the various substances present in the cells are shown diagrammatically in Text-fig. 2.

Since we have so little information as to the chemical natures of any of these substances, it is only possible to guess at the parts which they play in the continuous changes which constitute the life of the cell. It seems probable that the vacuoles

and chondriosomes provide surfaces suitable for many important chemical reactions (Robertson, 1926; Parat, 1928). Marston



TEXT-FIG. 2.

Diagram of the substances present in the neurons of the stellate ganglion of Cephalopods (neurofibrils omitted). *ch*, chondriosomes; *GB*, Golgi bodies; *K*, karyosome; *N*, nucleus; *NCt*, nucleus of connective tissue; *NS*, Nissl substance; *Pl*, plasmosome; *V*, vacuoles.

(1923) has shown that azine dyes (such as neutral red or janus green) combine with and precipitate proteolytic enzymes, and Koehring (1930) has argued that we may therefore use these dyes as indicators of the presence of such enzymes in living organisms. She has brought much evidence showing that the only fluids in the body which will stain with neutral red are known to contain proteolytic enzymes. If this reasoning is sound we may say that in many cells (including the neurons of Cephalopods) the proteolytic enzymes are isolated in vacuoles at whose surfaces they act: this would help to solve an old problem as to how such enzymes can exist free in the protoplasm without dissolving it. It would be unwise to build too much on such an argument, however, until it has been shown that the neutral-red reaction does not depend on physical concentration of the dye in a particular phase of the cell; a little evidence that the reaction is a chemical and not a physical one is given on p. 7. It is known (Matthews, 1930) that neutral red combines readily with phosphatides, and if the walls of the vacuoles are composed of lipins this might account for the staining reaction. A further indication that the vacuoles play a very important part in cell metabolism is given by the fact that the oxidation potential is higher around them than elsewhere in the cell (p. 14).

4. Nissl Substance.

(a) Observations of Normal Cells.

This substance has been reported as existing in invertebrate nerve-cells either in the form of separate 'grumes' (as in most vertebrate nerve-cells) or as a uniform mass of 'tigroid substance'. Among molluscs it has been studied by several workers in Pulmonates (Hanström, 1928; Kunze, 1921), in which it is said to consist of discrete granules lying round the nucleus and extending down into the axon. In Cephalopods, Garaieff (1909) reported that it consisted of a number of small separated bodies scattered through the cell, whereas Erhard (1912) gave a moderate figure of a mass of substance of foamy structure in a neuron of *Sepia*.

In preparations made with the methods given on p. 16 the Nissl substance is seen to lie as a homogeneous mass in the outer part of the cell (figs. 37-40, Pl. 5). It is not arranged in separate grumes (that is to say, it corresponds roughly to the 'gryochrome' type seen in the spinal ganglion cells of vertebrates), but in *Sepia* and *Loligo* it is somewhat divided up by the connective tissue which penetrates into the cells (figs. 37 and 38, Pl. 5). Except for these divisions the substance is evenly distributed over its whole area and extends into the first part of the axon. It is separated from the nucleus by a space, which in the living cell is occupied by the vacuoles, and the outlines of these latter can be seen, where they meet the Nissl substance (figs. 39, 40, Pl. 5). In gastropods (Kunze and Hanström, loc. cit.) the Nissl substance lies next to the nucleus, with the vacuoles outside it. These authors report that in *Helix* there is no axon hillock, and in fact that the Nissl substance extends into the axon. However, in a few preparations which I have made of the neurons of *Aplysia* (*limacina* and *depilans*) it seems that the Nissl substance is restricted to the cell-body (fig. 54, Pl. 6). Further, it consists of a homogeneous mass (gryochrome) and is not divided up into separate grumes.

Centrifuging ganglia for one hour at 3,500 revolutions per minute caused no apparent change in the distribution of the Nissl substance, vacuoles, or Golgi bodies. The only movement was of the karyosome which was found to shift readily inside the nucleus. This agrees with the observation of Brambell and Gatenby (1923) that no movement of the 'Golgi apparatus' is produced by centrifuging ganglia of *Helix*. It would seem, then, that the contents of the nucleus have a low viscosity, whereas that of the cytoplasm is very high, or else that all the substances in the cytoplasm have the same specific gravity (Cowdry, 1922). Probably the first alternative is correct, for no Brownian movement could be observed in the cytoplasm.

(b) Post-mortem Changes.

The changes which took place in the cells after death varied according to the conditions under which they were kept. When

the ganglia were removed from the body and left in a current of tank sea-water it was found that the cells underwent very little change during the first 15 hours.¹ If left for a longer period under these conditions the Nissl substance gradually disappeared so that the stain with toluidin blue became fainter and fainter. A mass of fine granules appeared in the nucleus and the karyosome became very small.

On the other hand, if the ganglia were left in the body, or under an inadequate circulation, very different changes were seen. After about 8-10 hours the perinuclear space became enlarged and the Nissl substance began to be more deeply stained on the side of the cell towards the axon and less stained at the opposite pole. Very distinct fibrils appeared in the cell-body and axon (fig. 42, Pl. 5). These processes continued, the Nissl substance apparently passing down into the axon, until finally the latter could be followed, deeply stained, for a considerable distance, whereas the cell-body appeared empty. At the same time the karyosome became smaller and granules appeared in the nucleus. These changes are shown in figs. 41-3, Pl. 5.

Further investigations showed that this movement of the Nissl substance was in some way connected with lack of oxygen. Stellate ganglia were cut out of the body of a freshly killed animal (*Octopus* or *Eledone*) and placed, one in 50 c.c. of ordinary tank sea-water and the other in 50 c.c. of sea-water from which all oxygen had been removed (see p. 7). After 24 hours under these conditions the ganglia were fixed and embedded and sectioned in a single block (to obtain sections of exactly equal thickness). It was found that the movement of the Nissl substance towards the axon was much more pronounced in the ganglion kept under anaerobic conditions than in that kept in normal sea-water.

In other experiments one ganglion was placed in sea-water plus potassium cyanide (making an N/1,000 solution) and the other in sea-water; both were fixed after about 24 hours. In the ganglia which had been in potassium cyanide the only stain of Nissl substance was in the axons, whereas in the control there

¹ The times vary very much with the temperature of the sea-water.

was a uniform, though rather pale, stain throughout the bodies of the cells. It seems, then, that lack of oxygen is at least one of the more important factors in determining this post-mortem migration of the Nissl substance.

As will be described below (p. 30) the movement of the Nissl substance occurred less rapidly if the connexion of the ganglion with the central nervous system had been severed before death.

No similar post-mortem changes in the Nissl substance have been previously reported. The changes observed have been vacuolization (Eve, 1896) chromatolysis (Piersanti, 1913), or simply the assumption of a diffuse appearance. Schreiber (1930) sometimes observed chromatolysis in the nerve-cells of Cephalopods following asphyxia, but no details are given.

IV. EFFECTS OF SECTION OF THE NERVES ON THE CELLS OF THE STELLATE GANGLION.

As stated above (p. 4) the exact connexions of the cells of the stellate ganglion are not yet known, nor have their ramifications been demonstrated histologically. With a view to solving this problem, Professor Sereni has made a number of experiments involving section of the mantle connective and stellar nerves, and he was good enough to hand over the material to me for histological examination.¹ The nerves were cut with fine scissors in anaesthetized animals; the operations are very slight and do not seriously affect the animals. In some cases operations for other purposes were performed simultaneously on the same animals, but these were not such as to alter the course of the changes in the nerves, except that they possibly affect the times taken by the changes.

An account of the degeneration and regeneration of the nerve-fibres will appear separately. The present account is concerned only with the changes in the cells of the stellate ganglion.

1. Section of the Mantle Connective.

In all, twenty-four ganglia have been examined (mostly from *Octopus* and *Eledone*, three from *Sepia*) after the mantle

¹ In addition, a certain number of extra experiments were made to fill the gaps in the series.

connective had been cut for periods varying from 1 to 171 days. In the cases in which the ganglia were examined directly after death, no differences could be observed, either in the Golgi bodies, vacuoles, or Nissl substance between the normal and operated sides. If, however, the ganglia were fixed some time after death, it was found that post-mortem changes set in much more rapidly on the normal than on the cut side. This was particularly noticeable in the case of the movement of the Nissl substance towards the axon (see p. 28), which was found to be well advanced in the cells of the normal ganglion, whereas the cells of the ganglion whose mantle connective had been severed remained intact. This difference manifested itself equally whether the mantle connective had been cut 1 hour or 40 days before death.

In order to demonstrate this effect two *Octopus* were taken and one mantle connective of the first was cut 1 hour before death, and one of the second 1 hour after death, which was produced by cutting open the mantle and removal of the heart and other viscera. The animals were left for 24 hours and the ganglia then fixed and sectioned. Whereas in the first case there was definitely more post-mortem change in the cells of the normal ganglion than in those whose mantle connective had been cut, in the second there was no such difference.

It seems that connexion with the central ganglia at the time of death causes more rapid onset of the post-mortem changes. Perfectly comparable differences in the physiological behaviour of the ganglia were constantly observed by Sereni (1929), who supposes that impulses pass down the mantle connective and, so to speak, tire out the ganglia, so that post-mortem change sets in more rapidly than in a ganglion isolated from such influence.

2. Section of the Stellar Nerves.

This operation was performed on twenty-nine *Eledone*, eleven *Octopus*, and seven *Sepia*, the times of survival varying from 1 to 164 days. The nerves were cut sometimes close to the ganglion and sometimes as far away as possible. The stellar nerves are accessible for only a short distance beyond

the ganglion, and usually they were cut at a distance of about 1 cm. from the latter. It is impossible to cut all the stellar nerves except close to the ganglion, and this was done only occasionally. Only ganglia fixed soon after death were studied.

(a) *Octopus* and *Eledone*.

During the first four days after section of the axons no change was observed in the cells of the stellate ganglion unless the cut had been made close to the ganglion; in which case there was complete disruption of some of the cells. At about the fifth day the Nissl substance of the affected cells assumed a uniform appearance, less granular than usual (fig. 45, Pl. 6), and soon afterwards it began to disappear from the central part of the cell. This change continued until nothing but a few isolated granules remained, scattered through the cells, with perhaps a very small mass near the nucleus. At the periphery the substance remained, but in the form of small lumps, separated by unstained spaces (figs. 46 and 47, Pl. 6). These changes reached their maximum between about the seventh and the fifteenth days, and they were seen both in cells whose axons had been cut near to the ganglion and in those cut farther away, being rather more marked in the former. There was no movement of the nucleus to the periphery of the cell, nor any apparent change in the karyosome.

After about the fifteenth day the Nissl substance began to re-form. It was not possible to determine exactly from what source it was derived, but in many cells a band of deeply staining substance could be seen in the middle of the cytoplasm, separated by clear spaces both from the nucleus and from the peripheral layer of Nissl substance (figs. 51-8, Pl. 6). This is about the position of the outer edge of the mass of vacuoles, and it seems likely that the Nissl substance is formed in connexion with them. In other cells it seemed to be re-forming chiefly at the periphery of the cell (figs. 48-50, Pl. 6). It seems certain that the Nissl substance is formed *in situ* in the cytoplasm and not by extrusion from the nucleus, or even in close connexion with the latter (cf. Heidenhain, 1911; Nicholson, 1928). During this period of regeneration, the Nissl substance was frequently

seen to be most densely concentrated on the side of the cell towards the axon (figs. 49 and 50, Pl. 6).

There is a gap in the series of animals studied between one killed 21 days and another 36 days after the operation. In the former the regeneration of the Nissl substance was well advanced, and in the latter (and subsequent) animals no differences between the operated and control ganglia were observed. Preparations of the stellar nerves and mantle muscles have shown that degeneration of the fibres peripheral to the cut is very rapid and is in fact complete within about 8 days. Regeneration begins at once but proceeds slowly, and is not complete for some months after the operation. Heidenhain (1911) supposed that the Nissl substance consisted of chromatin which served to maintain a normal nuclear-plasmatic ratio in nerve-cells, and that it therefore disappeared when part of the axon was cut off, and gradually re-formed as the new axon grew out, thus maintaining a constant N/P ratio. This cannot be so in the present case, since the re-formation of the Nissl substance was complete after about 30 days, when regeneration of the axons was still incomplete.

Besides the changes in the Nissl substance, those of the vacuoles and Golgi bodies were also studied. Some days after the operation the Golgi bodies (*sensu stricto*) became divided up to form a fine powder (fig. 30, Pl. 4) and then disappeared from the side of the cells nearest to the axons, leaving only the larger vacuoles (figs. 31-4, Pl. 4). This change also spread to the opposite pole of the cells, so that in extreme cases only a few large vacuoles remained. The exact significance of these changes is obscure, but at least they emphasize the difference between the large vacuoles and the rest of the complex, and especially the greater stability of the former.

In many cases it was observed that the neurofibrils were much more distinct in the cells whose axons had been cut than in normal cells. This was best seen in certain Da Fano preparations in which the network of neurofibrils was quite clearly stained in the affected cells, although it could not be seen at all in the uninjured cells of the same ganglion (fig. 35, Pl. 4).

None of the above-mentioned changes in the Nissl substance,

Golgi bodies, or vacuoles were seen in the smallest cells which lie next to the neuropil (fig. 32, Pl. 4). These are therefore presumably intercalary neurons whose axons do not extend out into the stellar nerves.

(b) *Sepia*.

The changes in the cells of the stellate ganglion of *Sepia* after section of the stellar nerves were somewhat different from those observed in *Octopus* and *Eledone*. In this case the nerves were always cut close to the ganglion, and this, together with the more rapid progress of all changes in *Sepia*, probably accounts for the differences.

In an animal killed 3 days after the operation the Nissl substance of the cells had assumed a coarse foamy appearance, and in some cells it had broken up into a number of large, deeply staining lumps (fig. 44, Pl. 5). These then disappeared, so that 6 days after the operation the cells had a uniformly pale aspect.

The changes in the Golgi bodies and vacuoles resembled those seen in *Octopus* and *Eledone*, except that the larger vacuoles, which were all that remained, moved to the periphery of the cells (fig. 36, Pl. 4). Since the cuts were made close to the ganglion, the traumatic degeneration extended into the neuropil. The degenerating tissue (including a mass of amoebocytes) became segregated within a capsule, with a wall several layers thick.

3. Conclusions from Section of the Nerves.

These experiments confirm existing ideas as to the connexions and functions of the stellate ganglion. Section of the mantle connective had no direct effect on the cells, indicating that no fibres run up to the central nervous system from the stellate ganglion (though some sensory fibres, running in this direction, presumably pass right through the ganglion). On the other hand, section of the stellar nerves caused retrograde degeneration of the cells of ganglion. It therefore appears that fibres running centrifugally in the mantle connective end in the stellate ganglion and that the axons of the latter run out in the stellar

nerves to the muscles. This hypothesis is confirmed by the fact that after section of the mantle connective retrograde degeneration is seen in the cells of the perioesophageal ganglia, though these changes have not been worked out in detail (Young, 1929).

4. Comparison of Retrograde Degeneration in Cephalopods and Vertebrates.

To the best of my knowledge there has been no previous investigation of retrograde degeneration in any invertebrate. The changes described above are very similar to those seen in vertebrates after section of the axons of nerve-cells. The disappearance of the Nissl substance from the centre of the cell and its conservation in lumps at the periphery are comparable even in details. In vertebrates it is common to find changes in the nucleus which becomes shifted to the periphery of the cell, while the karyosome becomes enlarged: no such changes were seen in Cephalopods. The changes in the Golgi bodies and vacuoles are difficult to compare, since the exact relationships of these structures in the neurons of vertebrates are still obscure. It is, however, well known (Penfield, 1922; Fananas, 1913; Cajal, 1914; Marcora, 1910) that after section of the axon the 'Golgi apparatus' becomes shifted to the periphery of the cell ('retispersion') and often completely disappears ('retisolution'). Penfield believes that the trophospongium of Holmgren does not become so dispersed, and if, as seems possible, this latter represents the vacuoles, its behaviour would be comparable to that of the larger vacuoles of the Cephalopods, which, as shown above, are very resistant to retrograde degeneration.

The conservation (and even marked increase in visibility) of the neurofibrils in the cells whose axons have been cut has also been observed in vertebrates, and in fact forms the basis of one of Donaggio's 'laws' of the behaviour of the neurofibrils (see Vizioli and Gozzano, 1930).

V. EFFECT OF STIMULATION OF THE MANTLE CONNECTIVE ON THE CELLS OF THE STELLATE GANGLION.

Many years ago Vas (1892), Lambert (1898), and Mann (1894) performed experiments demonstrating that the Nissl substance

disappeared from nerve-cells as a result of electrical stimulation or fatigue. These workers stimulated the cervical trunk of the sympathetic chain and studied the effects in the cells of the superior cervical ganglion. After about 15 minutes' stimulation the volume of the cell and of the nucleus was found to have increased and the Nissl substance to have moved away, from the centre of the cell. After further intermittent stimulation for 6-9 hours (Mann) the nuclei shrivelled and collapsed and the Nissl granules disappeared. The distance of the electrodes from the ganglion was 3 cm. (Vas), 'quelques centimetres' (Lambert), and unstated by Mann.

Lugaro (1895) partly confirmed these results, but Eve (1896), who repeated the experiments with more critical methods, failed to find any constant disappearance of the Nissl substance from the cells of the superior cervical ganglion after stimulation of the sympathetic trunk at a distance of 5 cm. Neither could he demonstrate any marked changes in the neurons after strychnine poisoning; the most extreme change seen was the assumption of a less granular, uniform, watery aspect. Holmes (1902), however, observed progressive disappearance of the Nissl substance after strychnine poisoning (frog), but Sereni (1921) was unable to confirm this result. The latter author supposes that during fatigue substances are produced in the nerve-cells and washed away by the circulation, giving the effect of chromatolysis. Since the circulation was continued in Holmes's experiments, but not in Sereni's, this would sufficiently explain the discrepancies. Several workers (see Carlson, 1903) have claimed that the Nissl substance in the cells of the retina decreases in amount after prolonged exposure to light.

The stellate ganglia of *Octopus* and *Eledone* constitute very suitable material for similar experiments, since the mantle connective can be stimulated as far as 6 cm. from the ganglion. The animals were killed by removal of the arms, the 'cranium' immediately opened and the peri-oesophageal ganglia cut out. All the viscera were then removed and the mantle laid out flat in a shallow glass dish. The tissues were kept moist throughout the experiment by the application of sea-water.

The mantle connective was dissected out on one side and

stimulated. In four experiments bipolar electrodes were used and placed below the nerve as far as possible from the ganglion (3-6 cm.). The shocks were given by means of an induction-coil with a relay arranged so as to give periodic shocks (varying from 90-120 per minute in different experiments). Relatively strong stimuli (coil distance 6-9 cm.) were used. It was found that even with weaker (minimal) stimuli the nerve itself became injured by these bipolar electrodes, so that the muscles of the mantle and the chromatophores soon ceased to respond unless the electrodes were moved nearer to the ganglion. This effect has been previously reported by Burian (1907). In three other experiments, therefore, a single fine electrode was placed under the nerve, and the other electrode, a thick wire, was tied into the muscles of the funnel, in the middle line. When the nerve was stimulated in this way the muscles continued to respond for as much as 50 minutes, but finally ceased unless stronger stimuli were employed, in which case there was a temporary return of the contractions, but these died away again in the course of a few minutes. After all response of the muscles to stimulation of the mantle connective had ceased, it was found that stimulation of the ganglion itself, or of the stellar nerves, still caused strong contraction, showing that there is a synapse in the ganglion which tires first and that the fatigue does not lie in the myoneural junction or muscles themselves.

In a third group of two experiments bipolar electrodes were placed under one mantle connective as near as possible to the ganglion itself in order to test the effect of escape of the current to the cells themselves. In this case contractions continued for as much as 90 minutes, but eventually died away, probably on account of local damage to the ganglion, since stimulation of the hind part of the latter, or of the stellar nerves, still caused contraction.

The time of stimulation varied in different experiments from 80-117 minutes; usually without interruption, but occasionally with one or two short periods of rest. Stimulation was always continued for a long time after all response of the muscles had ceased. The chromatophores continued to expand (that is to say, their muscles contracted) for much longer periods than the

mantle muscles, and this is in accordance with the fact, now well established (Sereni, 1929), that the nerves to the chromatophores run through the stellate ganglion without synapse.

At the end of each experiment the two stellate ganglia were fixed. Usually each ganglion was divided into two halves, and of these one fixed for the study of the Nissl substance and the other with Da Fano's fluid for the vacuoles and Golgi bodies. The stimulated and control pieces were treated exactly alike, embedded in the same wax-block and cut together, so as to ensure sections of equal thickness.

In no case was there any marked difference between the Nissl substance or Golgi bodies of the normal and stimulated ganglia. In two cases (stimulated far from the ganglion) it seemed that the Nissl substance was more granular on the cut side and slightly aggregated into small lumps at the periphery of the cell. The differences were very slight, however, and even after considerable study the normal and stimulated ganglia could not be identified by the appearances of their cells. A number of measurements of the size of the nucleus and karyosome were made, but no significant differences in size were revealed.¹ After one experiment pieces of the normal and control ganglia were stained *intra vitam* with neutral red; no differences in the vacuoles could be discerned.

After the experiments in which the electrodes were placed close to the ganglion it was noticed that the nuclei of the connective tissue and neuroglia stained much more deeply than did those of the normal ganglion.

It must, therefore, be concluded that stimulation of the mantle connective for the period stated does not cause any marked change in the nucleus, Nissl substance, Golgi element, or vacuoles of the neurons of the stellate ganglion, even when the current is allowed to escape to the ganglion itself. Possibly intermittent stimulation over a longer period would reveal some changes, but it is impossible to stimulate the preparation continuously via the mantle connective for more than a limited period, since the synapse in the ganglion rapidly becomes

¹ I am indebted to Mr. E. B. Ford for assistance in making the necessary calculations.

fatigued. It would, however, be interesting to repeat the experiments with an intact circulation, since the absence of chromatolysis may be due to the lack of circulating blood to wash away the substances produced by the fatigue.

SUMMARY OF CONCLUSIONS.

1. The neurons of the stellate ganglia of Cephalopods are surrounded by connective tissue which penetrates into canals in the cytoplasm. The nuclei in these canals belong to the connective tissue.

2. The nuclei of the neurons contain a fluid of rather low viscosity, in which are suspended a basophil karyosome, one or more oxyphil plasmosomes, and a number of smaller granules.

3. Around the nucleus there is a mass of bodies, visible in the unstained cells, which stain readily with neutral red, and have been shown to have a definite outer membrane which is permeable to water but impermeable to inorganic ions. These vacuoles maintain their identity when squeezed out of the cells.

4. The vacuoles stain somewhat with osmium tetroxide both when immersed directly in a 0.5 per cent. solution in sea-water and after Kolatschev's method. They often stain deeply with silver after Cajal's and Da Fano's methods.

5. After staining with Kolatschev, Cajal, or Da Fano's techniques a number of granules can be seen in the neighbourhood of the vacuoles. They usually (perhaps always) lie near the surface of the latter, but certainly do not constitute their outer membrane. Not all of the vacuoles are accompanied by such bodies. The term Golgi bodies (or substance) is restricted to these granules.

6. The chondriosomes are smaller bodies scattered throughout the cell; they were not found to stain with osmium or silver.

7. Centrifuging the ganglia produced shifting of the karyosome within the nucleus, but no changes in the positions of any of the substances in the cytoplasm.

8. The region of the vacuoles has been shown to have a higher oxidation potential than the rest of the cell.

9. The Nissl substance forms a homogeneous mass in the outer part of the cell and is not divided up into separate grumes;

it extends into the axon. It undergoes peculiar post-mortem changes when kept under anaerobic conditions.

10. The Nissl substance of *Aplysia* lies round the nucleus. It is not divided up into grumes and does not extend into the axon.

11. After section of the mantle connective no changes were seen in the Nissl substance of the stellate ganglion, though the cells were shown to be in an abnormal physiological condition.

12. After section of the stellar nerves marked retrograde degeneration was seen in the cells of the stellate ganglion, and this is described in detail. The Nissl substance later regenerates *in situ*, and not in connexion with the nucleus; possibly it is formed in connexion with the outer layer of vacuoles.

13. Electrical stimulation of the mantle connective caused no marked changes in the nuclei, Nissl substance, or Golgi bodies of the cells of the stellate ganglion.

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DESCRIPTION OF PLATES 1-6.

All the figures are of cells from the stellate ganglion. For details of technique, &c., see the tables following.

ABBREVIATIONS.

A, axon; *AH*, axon hillock; *C*, chondriosomes; *Cr*, crystals deposited with p-phenylenediamin; *Ct*, connective tissue; *GB*, Golgi bodies; *GB'*, Golgi bodies during dispersal; *I*, deposits produced by indophenol-blue reaction; *IN*, intact intercalary neuron; *K*, karyosome; *N*, nucleus of neuron; *Nct*, nucleus of connective tissue; *NS*, Nissl substance; *NS'*, Nissl substance regenerating; *NS''*, lumps of degenerating Nissl substance; *P*, plasmosome; *V*, vacuole; *V'*, space left by dissolved vacuole; *X*, stained mass (p. 23).

PLATES 1 and 2.

All figures are camera lucida drawings made with a Zeiss apochromatic oil-immersion objective (90. 1.3. 2 mm.).

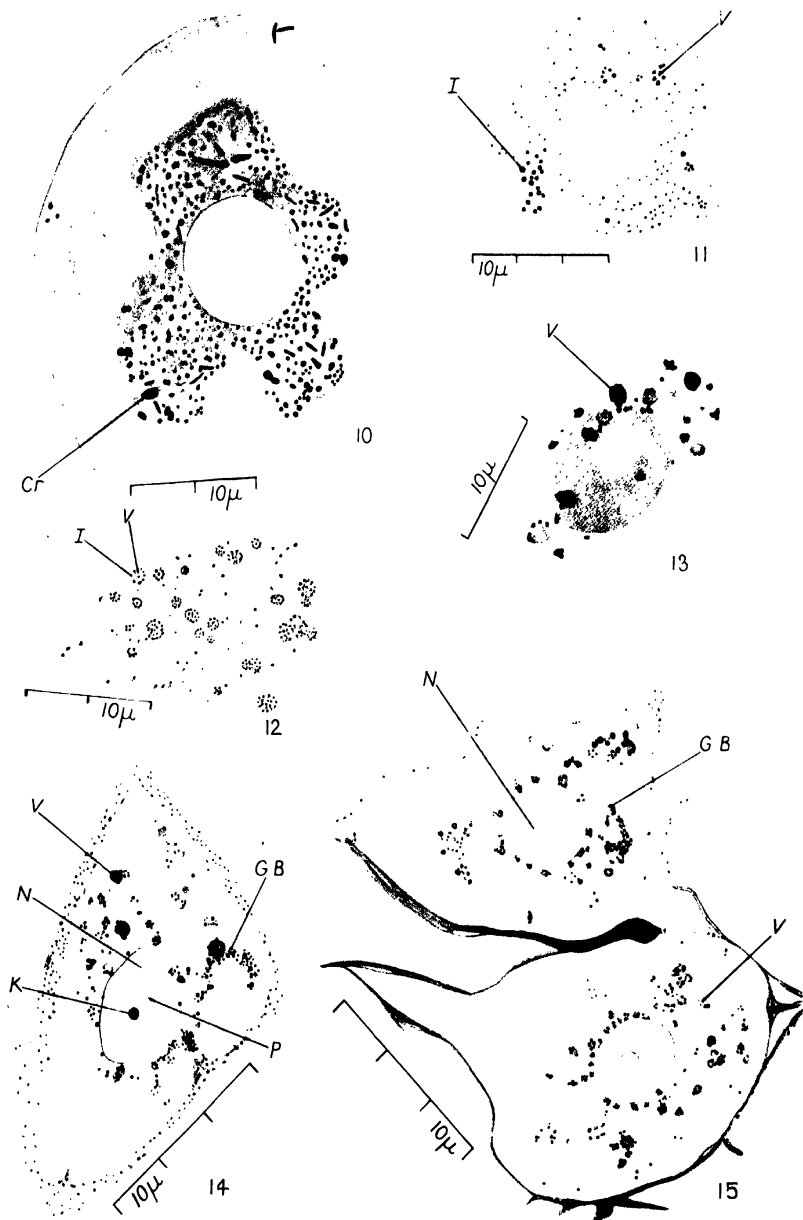
<i>Fig.</i>	<i>Species.</i>	<i>Substances shown.</i>	<i>Technique.</i>	<i>Ocular.</i>
1	<i>Sepia officinalis</i>	Vacuoles	Neutral red, 1/50,000	K 6
2	" "	"	" "	K 6
3	<i>Eledone moschata</i>	Vacuoles and Golgi bodies	Kolatschev, acid fuchsin. Sections 6 μ thick	K 10
4	<i>Octopus vulgaris</i>	Vacuoles	15 min. in neutral red 1/40,000 dissolved in sea-water + 3.5 per cent. NaCl.	K 20
5	" "	"	15 min. in neutral red 1/100,000 in sea-water + 7 per cent. NaCl.	K 20
6	<i>Sepia officinalis</i>	"	Freezing-section stained neutral red 1/50,000	K 20
7	" "	Vacuoles and crystals	Neutral red 1/50,000 for 30 min.; p-phenylenediamin 0.1 per cent. for 25 min.	K 20
8	" "	Vacuoles and crystals	Neutral red 1/50,000 for 20 min.; p-phenylenediamin 0.1 per cent. for 40 min.	K 7
9	" "	Vacuoles and chondriosomes (?)	Neutral red 1/50,000 for 20 min.; janus green B 1/5,000 for 30 min.	K 20
10	" "	Crystals	P-phenylene-diamin 0.1 per cent. for 2 hrs.	K 10
11	<i>Octopus vulgaris</i>	Vacuoles, &c.	'Indophenol - blue' reaction for 30 min.	K 6
12	<i>Sepia officinalis</i>	"	'Indophenol - blue' reaction for 30 min.	K 10
13	" "	"	Methylene blue 1/10,000 for 25 min.; ammonium molybdate 8 per cent.	K 20
14	<i>Eledone moschata</i>	Vacuoles and Golgi bodies	Kolatschev. Section 6 μ thick	K 10
15	<i>Octopus vulgaris</i>	Vacuoles and Golgi bodies	Da Fano. Section 3 μ thick	K 10

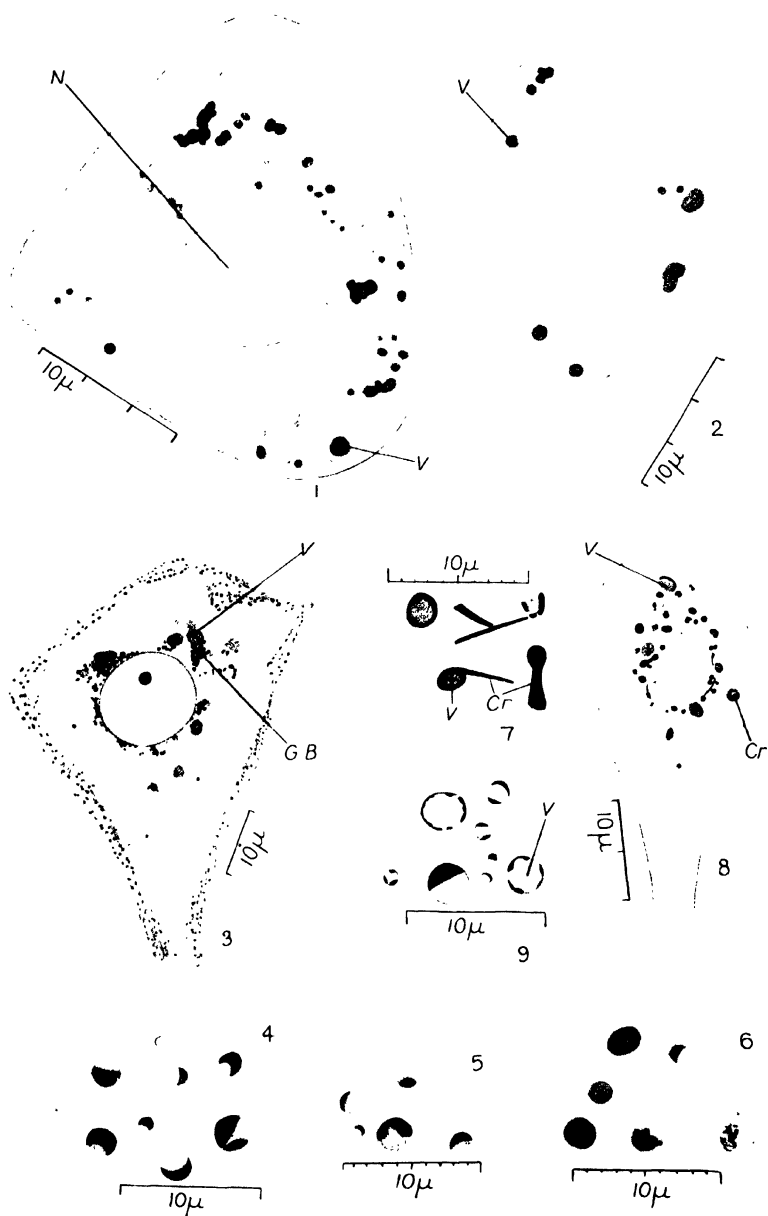
PLATES 3-6.

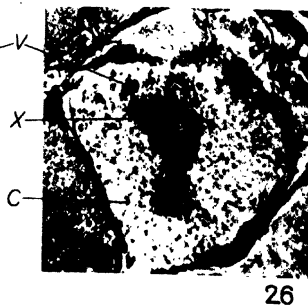
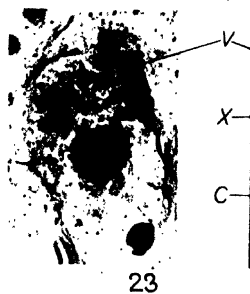
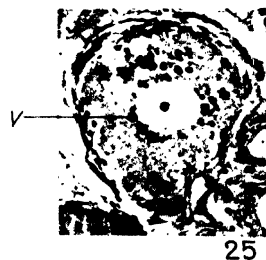
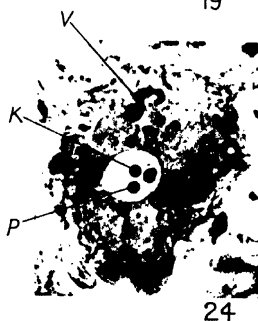
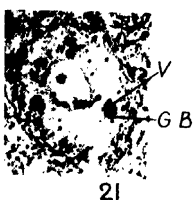
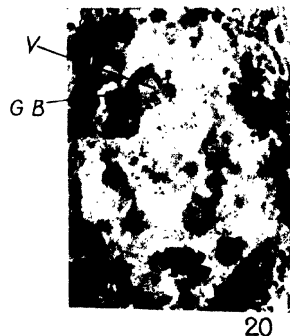
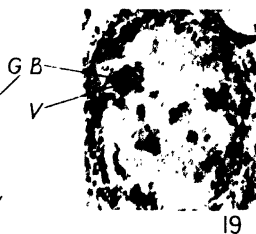
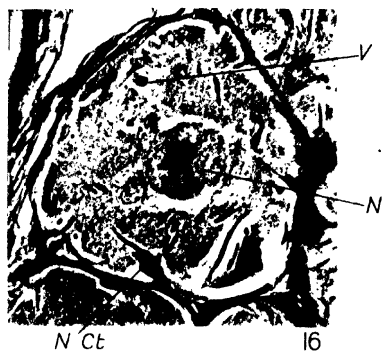
All the figures are untouched photographs taken with a Zeiss apochromatic oil-immersion objective (90. 1.3. 2 mm.).

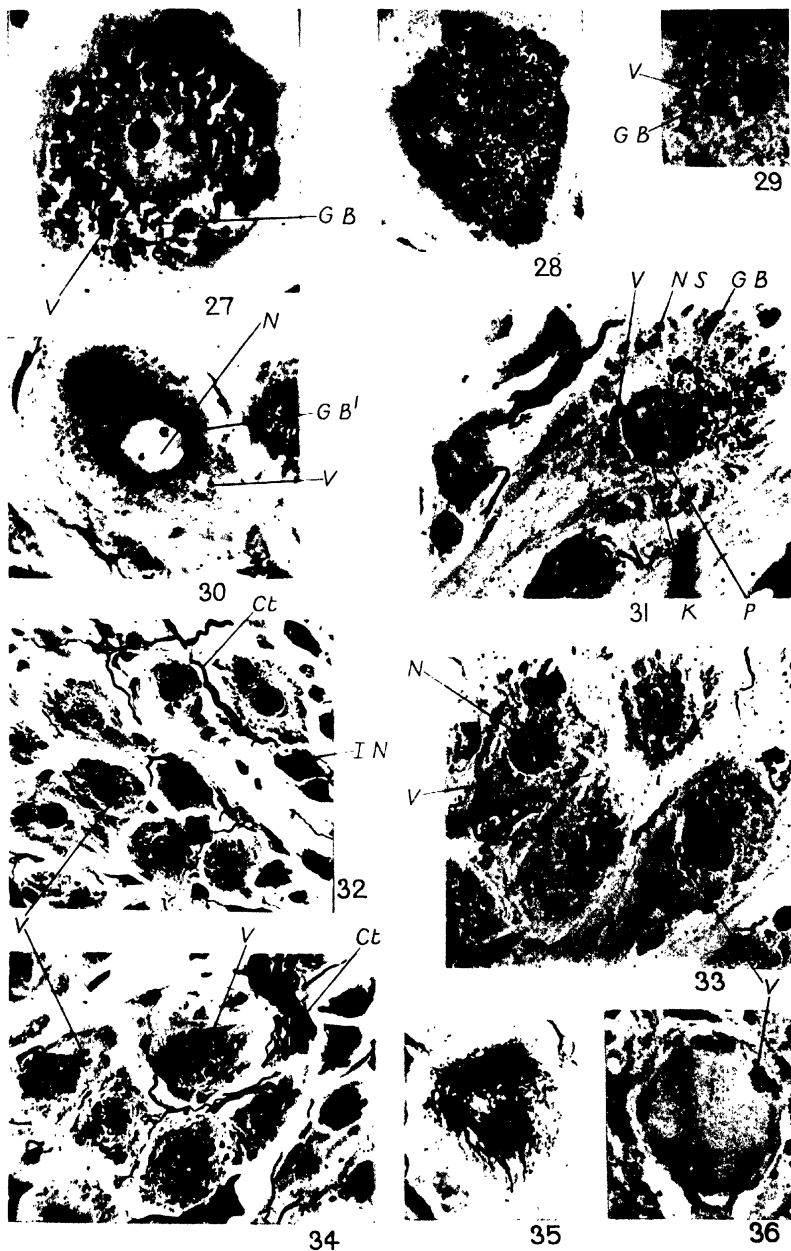
<i>Fig.</i>	<i>Species</i>	<i>Operation.</i>	<i>Substances shown.</i>	<i>Technique.</i>
16	<i>Sepia officinalis</i>	None	Ingrowths of connective tissue	Regaud; iron haematoxylin
17	<i>Sepia officinalis</i>	..	Vacuoles	Regaud; iron haematoxylin
18-21	<i>Eledone moschata</i>	..	Vacuoles and Golgi bodies	Kolatschev
22-3	<i>Eledone moschata</i>	..	Vacuoles	Cajal uranium nitrate
24-5	<i>Octopus macropus</i>	..	Vacuoles and plasmosomes	Champy; iron haematoxylin
26	<i>Sepia officinalis</i>	..	Chondriosomes	Regaud; iron haematoxylin
27	<i>Sepia officinalis</i>	Control of fig. 36	Golgi bodies, vacuoles, Nissl substance	Da Fano, Ehrlich's haematoxylin
28	<i>Eledone moschata</i>	Control of figs. 31-4	As fig. 27	As fig. 27
29	<i>Octopus vulgaris</i>	None	Vacuoles and Golgi bodies	Da Fano
30	<i>Eledone moschata</i>	Stellar nerves cut 6 days	Vacuoles and Golgi bodies	..
31-4	<i>Eledone moschata</i>	Stellar nerves cut 18 days	As fig. 28	As fig. 28
35	<i>Octopus vulgaris</i>	Stellar nerves cut 9 days	Neurofibrils	Da Fano
36	<i>Sepia officinalis</i>	Stellar nerves cut 8 days	Vacuoles	..
37	<i>Loligo vulgaris</i>	None	Nissl substance	Carnoy
38	<i>Sepia officinalis</i>
39-40	<i>Eledone moschata</i>	95 per cent. alcohol + 2.5 per cent. HNO ₃
41	<i>Eledone moschata</i>	Fixed 20 hrs. after death	95 per cent. alcohol
42	<i>Eledone moschata</i>	Fixed 20 hrs. after death	Nissl substance, neurofibrils
43	<i>Eledone moschata</i>	Fixed 24 hrs. after death	Nissl substance

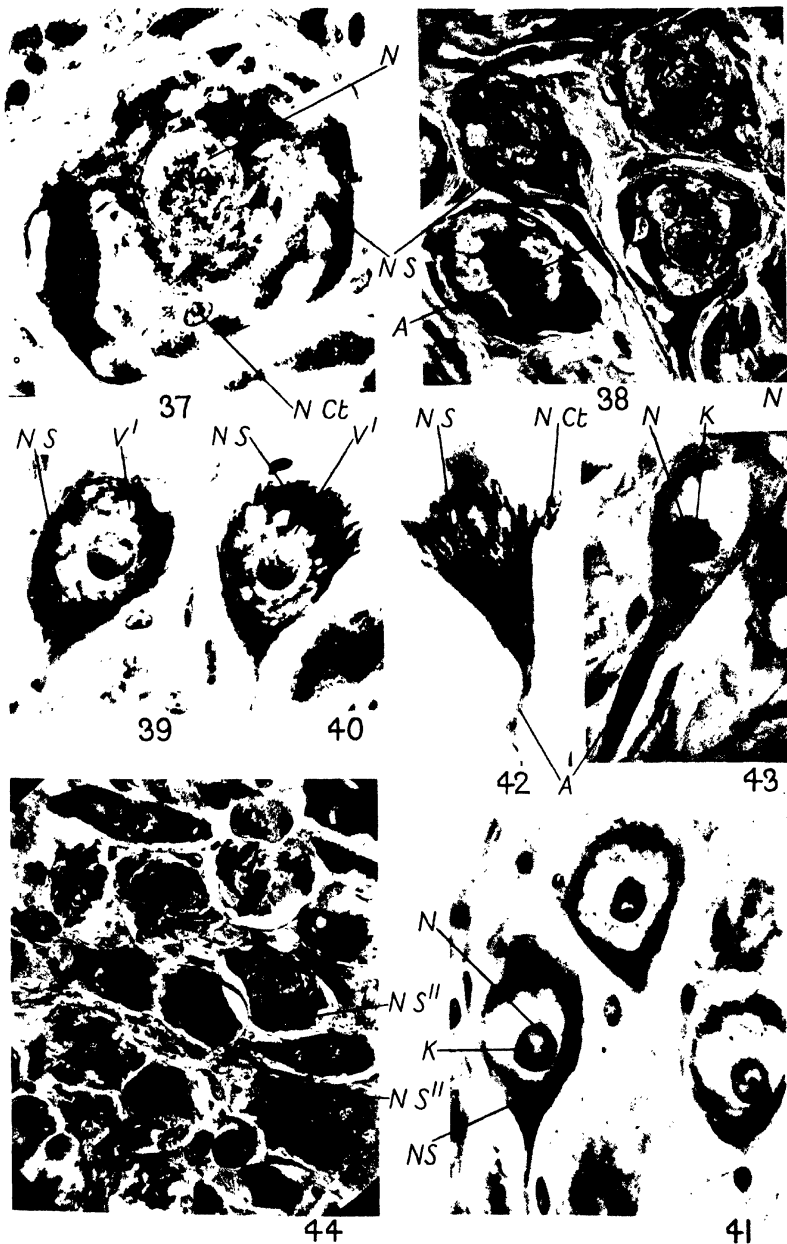
<i>Fig.</i>	<i>Species.</i>	<i>Operation.</i>	<i>Substances shown.</i>	<i>Technique.</i>
44	<i>Sepia officinalis</i>	Stellar nerves cut 4 days	Nissl substance	Carnoy
45	<i>Eledone moschata</i>	Stellar nerves cut 7 days	„ „	95 per cent. alcohol +5 per cent. HNO_3
46	<i>Eledone moschata</i>	Stellar nerves cut 7 days	„ „	Carnoy
47	<i>Eledone moschata</i>	Stellar nerves cut 9 days	„ „	„
48-9	<i>Eledone moschata</i>	Stellar nerves cut 17 days	„ „	95 per cent. alcohol +5 per cent. HNO_3
50	<i>Eledone moschata</i>	Stellar nerves cut 17 days	„ „	95 per cent. alcohol +3 per cent. HNO_3
51-3	<i>Eledone moschata</i>	Stellar nerves cut 21 days	„ „	Carnoy
54	<i>Aplysia limacina</i>	None	„ „	95 per cent. alcohol +3 per cent. HNO_3

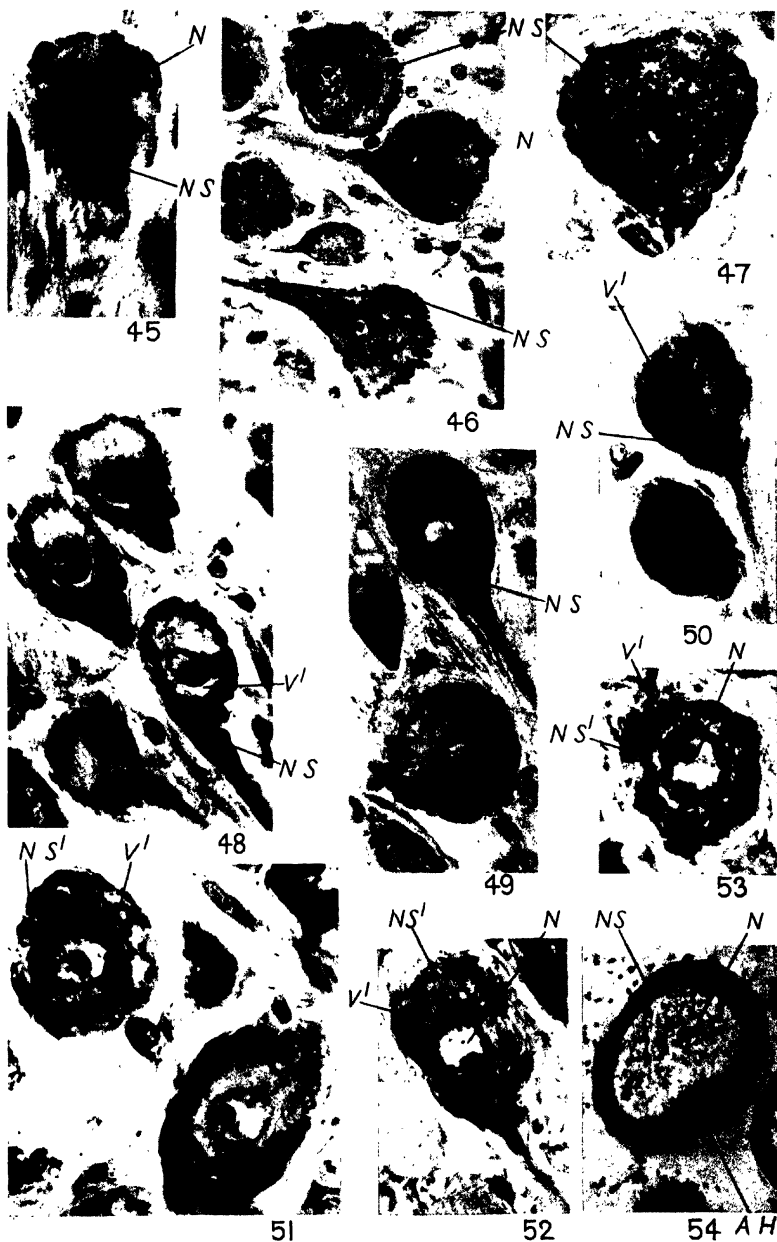












The Structure and Development of the Reproductive System in the Coleoptera with notes on its Homologies.

By

Margot E. Metcalfe, Ph.D.

With Plates 7 to 10 and 49 Text-figures.

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PART I. THE MALE.

A. INTRODUCTION.

WHILE a great deal of valuable literature upon the morphology and development of the reproductive system in the Insecta is in existence, investigators in the past have made but little attempt to compare their conclusions with those of other workers. There are in existence, therefore, different systems of nomenclature for almost every group studied, often more than one system for the same group, and always a different system for each of the sexes. In consequence, a bewildering number of terms is in use, these being often of popular designation and so of little systematic value.

If the morphological structure of the reproductive organs, and particularly of the external genital appendages, is to be of any phylogenetic significance, it is necessary that the number of terms in use should be reduced to a minimum, and that the selection of terms should be based upon a study of comparative morphology. There is a growing feeling among students of taxonomy and phylogeny that the importance of the reproductive organs has been too long ignored, and attempts to reorganize systems of classification upon this basis have been begun.

In the most recent work upon this subject, such as that of Crampton (7-15), Walker (55 and 56), and Singh Pruthi (42-5), although the conclusions of these authors are not entirely in agreement, much has been done in the reviewing and criticizing of earlier work, towards the establishment of the subject upon a firmer basis.

With regard to the Coleoptera, Verhoeff (49 and 50) has made a survey of the terminal abdominal segments and their appendages in both sexes, while Sharp and Muir (47) have studied the copulatory apparatus in a large number of species. The only authors who have treated the subject from the standpoint of development, however, appear to be Muir (34) and Singh

Pruthi (44 and 45), and the following study is an attempt to supplement their work.

The first and second parts will be devoted to the structure and development of the male and female respectively, the third section to a comparison between the two sexes.

The work was carried out in the Department of Zoology, University College of Wales, Aberystwyth, under the supervision of Professor R. D. Laurie, M.A., to whom the writer is greatly indebted for much valuable advice and criticism. Many thanks are due to Mr. J. R. W. Jenkins, M.Sc., for his unfailing interest and encouragement. The writer also wishes to express her gratitude to the Department of Scientific and Industrial Research for a Grant enabling the research to be carried out.

B. MATERIAL AND TECHNIQUE.

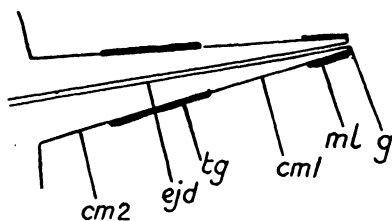
Sitodrepa panicea L. was obtained from samples of cattle-cake which had become infested while stored in the laboratory; *Gastroidea polygoni* L. was reared from eggs found on weeds of *Polygonum* spp. during June and July. Larvae were preserved at different stages of development, and when the fully-fed larvae had left the plants prior to pupation, the soil was carefully sieved for the pupae at intervals of seven and fourteen days. *Anthonomus pomorum* L. was first obtained from 'capped' blossoms which were brought into the laboratory for examination towards the end of May. Further specimens were obtained from Cambridgeshire and Devonshire. *Rhagium bifasciatum* F. was collected from rotten stumps of coniferous trees. Larvae in different stages of development were obtained throughout the year, but the larval period extends over more than one year, and, pupation taking place in late summer and early autumn, the adult emerges almost immediately. Of the pupae obtained and successfully examined all but one proved to be female. The account of the male is, therefore, unavoidably held over.

The adult insects were examined after dissection under the binocular microscope. Larvae and pupae were preserved in Carnoy's fluid and serial sections prepared in the customary manner.

C. GENERAL STRUCTURE AND NOMENCLATURE.

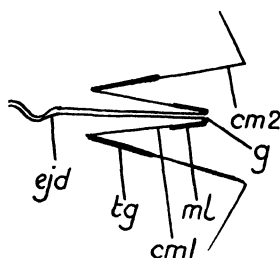
Before entering into a discussion on the male reproductive system, a brief description of the general structure in the Coleoptera is necessary. The terms used are selected from the systems of nomenclature employed by Sharp and Muir and Singh Pruthi.

A pair of testes occupies a dorso-lateral position, one on each



TEXT-FIG. 1.

Aedeagus extended.



TEXT-FIG. 2.

Aedeagus retracted.

cm 1, 1st connecting membrane; *cm 2*, 2nd connecting membrane; *ejd*, ejaculatory duct; *g*, gonopore; *ml*, median lobe; *tg*, tegmen.

side of the alimentary canal, extending through one or more of the abdominal segments between the first and the seventh. From each testis a fine duct, the vas deferens, leads posteriorly to open with its fellow of the opposite side, into the ejaculatory duct. At the point where vasa deferentia and ejaculatory duct unite, one or more pairs of accessory glands open, either into the vasa deferentia themselves or into the ejaculatory duct. A pair of vesiculae seminales may be present as dilations of the vasa deferentia, or there may be a common vesicula into which they both open.

The copulatory apparatus consists of an invagination of the body-wall known as the 'aedeagus' (Sharp and Muir) or the 'genital pocket' (Singh Pruthi) (Text-figs. 1 and 2). In structure it consists of a pair of tubes, the one within another, the walls of the inner and outer tubes being continuous at the orifice of the inner tube. The outer tube represents the wall of the

genital pocket, the inner the ejaculatory duct. This structure readily lends itself to modification. Traction on the ejaculatory duct will withdraw the genital pocket wholly within the body cavity; this is the normal position in repose (Text-fig. 2). The orifice of the genital duct thus becomes proximal, while the orifice of the genital pocket so formed is distal. In the act of copulation pressure exerted within the genital pocket results in its being protruded from the body-cavity as shown in Text-fig. 1.

Having pierced the tip of the genital pocket, the ejaculatory duct enters a median appendage at its base, the gonopore being situated at the apex. This appendage is known as the 'median lobe' (Muir, Singh Pruthi), though Verhoeff terms it the 'penis'.

In some Coleoptera, e.g. *Tenebrio molitor*, a pair of 'lateral lobes' (Singh Pruthi) or 'parameres' (Verhoeff) is present, one on each side of the median lobe. The lateral lobes may fuse with each other basally in the mid-dorsal and mid-ventral lines, thus surrounding the proximal region of the median lobe. When all three lobes are present, the copulatory apparatus is said to be of the 'generalized trilobe type'.

It is usual for a ring-like sclerite to be formed in the wall of the genital pocket by the secretion of chitin. The sclerite is known as the tegmen and may be formed in the outer or the inner wall of the pocket. The remainder of the wall being membranous, this allows of a certain amount of play between the tegmen and the median lobe which enables the latter to be extruded during copulation.

Sharp and Muir distinguish two membranous regions in the genital pocket, namely, the first connecting membrane between the tegmen and the median lobe, and the second connecting membrane between the tegmen and the body-wall. In a subsequent paper Sharp (48) reverses this order, the first connecting membrane becomes the second and vice versa. This, of course, does not alter the relations that exist between the several parts of the copulatory apparatus, and for that reason the earlier system has been retained in this paper.

The value of naming these divisions of the genital pocket as though they were distinct, fixed regions is doubtful, as Sharp

and Muir themselves realize. It tends to foster the idea that the genital pocket is a jointed tube composed of a number of definite sclerites. The tegmen, in point of fact, is not fixed in position at all. In some forms, e.g. various Curculionids, the chitin is deposited in the outer wall of the pocket to form a ring-shaped sclerite, and both first and second connecting membranes can be distinguished. In other forms, e.g. *Tenebrio molitor*, the first connecting membrane, i.e. between the tegmen and median lobe, disappears completely, and the tegmen now appears as the tubular 'basal piece'; the term tegmen being here applied by Sharp and Muir to the tegmen proper together with the median lobe. In the present paper, the term 'genital pocket' will be used to designate the whole of that invagination of the body-wall, while by 'tegmen' will be meant either the ring-like sclerite or the 'basal piece'.

The spiculum gastrale is a Y-shaped or horseshoe-shaped chitinous structure, or simple chitinous rod, lying against the ventral body-wall in the posterior segments of the abdomen. It serves as the basis of attachment for the powerful muscles which control the copulatory apparatus.

The male reproductive system may, therefore, be considered under two headings:

- (1) The External Organs or Copulatory Apparatus, consisting of the genital pocket, with the tegmen, connecting membranes, median and lateral lobes, and spiculum gastrale.
- (2) The Efferent System, comprising the paired testes, vasa deferentia, accessory glands, vesiculae seminales, and ejaculatory duct.

D. HISTORY OF THE SUBJECT AND HOMOLOGIES.

(1) The External Organs.

While the intromittent organ in the Insecta, i.e. the organ in the Coleoptera formed by the median and lateral lobes, has been shown by many authors to arise from originally paired appendages, e.g. Christophers in the Diptera (17) and *Cimex lenticularis* (16), Verson and Bisson in *Bombyx mori*

(54), Haviland (21), Kraepelin (28), Kulagin (30), Michaelis (31) in the Hymenoptera, Zander in the Hymenoptera (59), Trichoptera (60), and Lepidoptera (61), Singh Pruthi (43), George (20) in the Homoptera; Muir and Singh Pruthi are the only authors who have studied its development in the Coleoptera. The latter is of the opinion that the intromittent organ in this order also is paired in origin, but Muir states that it arises as a median, unpaired structure.

If Muir is correct, and the intromittent organ is unpaired in origin, then the Coleoptera form a unique order, the systematic position of which must be carefully revised. But if, as Singh Pruthi states, the intromittent organ originates as a pair of appendages of the ninth sternite, then the question of the homologies of these structures opens up an important line of investigation.

In general, the intromittent organ consists of a median appendage, variously termed the median lobe (Singh Pruthi), penis (Verhoeff, Zander, &c.), phallosome (Christophers), with one or more pairs of appendages on either side. The lateral appendages may be termed inner and outer valves (Zander), parameres (Verhoeff, Muir, Walker, &c.), lateral lobes (Singh Pruthi), androapodite and parameres (Christophers) as well as many other confusing names. In some cases the median appendage is double, i.e. there are two 'penes' as in some Dermaptera and the Ephemeroptera.

The difficulties which exist in determining on one system of nomenclature are numerous and formidable. The chief seems to lie in the recognition of an ancestral type of structure to which the parts in the different orders can be referred. Verhoeff (52) in 1903 laid down the foundations for a truer understanding of the relations of the parts. In this study, he gives a detailed account of the structure of the intromittent organ in the primitive Thysanuran *Machilis*, and attempts to refer Insects of higher orders to the same basic plan. Subsequent research by himself as well as by other authors has shown that Verhoeff was mistaken in many points. The work, however, is important and throws considerable light on the subject. Verhoeff considered that the genital appendages in *Machilis* are serially

homologous with the appendages found on the preceding abdominal segments and also with the thoracic legs and trophi (mouth parts). The sternites of the eighth and ninth segments are reduced in size, and the appendages, each consisting of a basal joint or coxite (gonocoxite) which bears an inner appendage, the telopodite, and an outer appendage, the stylus, are situated laterally. The whole appendage has been homologized with the crustacean limb, the coxite representing the basipodite, the telopodite the endopodite, and the stylus the expodite.

According to Verhoeff, there is a tendency for the coxites to become closely united with the sternites, eventually losing their identity and forming with the sternite a 'coxosternum'. Walker shows that such a fusion also occurs in the Orthoptera and that there is a progressive diminution of the styli with their ultimate loss. Verhoeff at first considered that the median appendage is formed by the telopodites of the eighth segment, and he names this organ the 'syntelopodite' or 'penis'. The telopodites of the ninth segment are termed 'parameres'. Later, he stated that the penis does not arise from the eighth segment, but from a segment posterior to the ninth (53). The term paramere then, in its original application, refers to the telopodites or endopodites of the ninth sternite, and in this connexion will be used hereafter.

Verhoeff believed, therefore, that the 'penis' and parameres do not belong to the same segment. Furthermore, that in such cases where a pair of penes exists, e.g. in the Ephemeroptera, and Dermaptera-Diandria, each of these penes is developed from a single telopodite. In the Dermaptera-Monandria, where only one penis is present, this does not represent a syntelopodite, but is the result of the atrophy of one of the pair, and the development of the other, as has been shown by many authors.

More recent workers, with the notable exceptions of Muir and Kershaw (27) and Wheeler (57), whose work will be discussed in Part III of this study, consider that the median and lateral appendages arise from the ninth segment only. To which of the appendages in the primitive insect *Machilis*, then, can they be compared?

Intromittent organs may be grouped roughly in two classes:

(a) With a median, and two pairs of lateral appendages.

(b) With a median, and one pair of lateral appendages.

The former group includes the Ephemeroptera, Orthoptera, Homoptera, some Lepidoptera, Trichoptera, some Hymenoptera, and Diptera.

Several possible comparisons may be made:

(i) That the median appendage is a syntelopodite, i.e. it represents the fused parameres, while the inner pair of appendages represents the coxites and the outer pair the styli.

(ii) That the median appendage and the inner lateral appendages are derived from the parameres, while the outer pair represents the coxites and/or their styli.

Walker considers that the penis or median appendage itself is not paired in origin. He says (55, p. 2): 'The aperture is usually borne upon an outgrowth, the penis or aedeagus, whose walls may be more or less chitinated, or wholly membranous. Where two apertures are present there are likewise two penes (Ephemerida) or a more or less deeply bi-partite penis (Dermaptera), but it is more probable that in these orders the penis or penes are not strictly homologous with those of other orders; in fact, it appears as though the penis may have developed independently in several orders.'

He therefore compares the two pairs of appendages present with the parameres and the coxites, with, or without their styli.

Crampton (14) believes that the median appendage is paired in origin and that the double penes in the Ephemerida represent the paramer; coxites and their styli also being present.

Zander (59-61), while not expressing an opinion as to the homologies of the various appendages, possibly because he does not consider them to be derived from abdominal 'limbs', regards the 'penis' and 'inner and outer valves' as derived from one pair of primary appendages. Christophers (17) also subscribes to Zander's account of the development of the appendages, but seems to consider the penis or phallosome as partially derived from a median outgrowth posterior to the ninth sternite.

According to Singh Pruthi (43), there are two pairs of primary

appendages in the Homoptera, the inner pair of which gives rise to both parameres and penis, while the outer pair represents the coxites. He also states that from Zander's description, he regards the same state of affairs to be present in the Lepidoptera, Trichoptera, and Hymenoptera, where a penis and two pairs of appendages are present. In these orders, where the penis and one pair of appendages only are present, he is of the opinion that the penis represents the parameres, and the lateral appendages the coxites, with or without their styli. The penis in such a case is called by Singh Pruthi an 'aedeagophore'.

George (20), working on the Homoptera homologizes the subgenital plates with the coxites, and states that the aedeagus and 'so-called parameres' are derived from the endopodites. He regards the parameres as organs of no morphological significance which arise as outgrowths from the aedeagus.

The second group includes the Dermaptera, Odonata, and Coleoptera. Here there are a median appendage and but one pair of lateral appendages, or, as in some Coleoptera, a median appendage only. These may have been derived in either of the following ways:

- (i) The median appendage from the parameres, the lateral appendages from the coxites,
- (ii) The median and lateral appendages together from the parameres.

On account of the position of the median and lateral appendages, it is considered unlikely that both should have been derived from the coxites.

In the Odonata, parameres are absent, and the lateral appendages, which bear styli in the nymphal instars, are thought to represent the coxites (George). This reduction of the genitalia associated with the ninth segment is correlated with the development of secondary structures having a copulatory function and situated in the anterior region of the abdomen. The lateral appendages in the Dermaptera represent the parameres (Crampton, Walker, Singh Pruthi).

The lateral appendages in the Coleoptera were at first declared by Muir (34) to be merely outgrowths from the aedeagus. Later, however (38), he suggested that they might be the

homologues of the coxites. To lend weight to this suggestion he writes: 'In some Coleoptera, a distinct pair of lobes is found arising from the base of the median lobe, quite independent of the lateral lobes (coxites) on the tegmen.'

From this quotation one might infer that this 'distinct pair of lobes' is the homologue of the pair of parameres in the Trilobe forms. There is, however, a distinct similarity between the above account and Zander's description of the Trichoptera-Limnophilidae, where the gonopore is situated on the median of three 'endäste', and a pair of lateral 'valvae' is also present.

Singh Pruthi is of the opinion, however, that coxites are not present in the Coleoptera, but that the median and lateral appendages together are homologous with the parameres. Where only the median appendage is present, the structure is termed an aedeagophore and represents the fused parameres. The discussion of this interesting problem will be returned to later.

Owing to absence of chitinization, the abdominal segments posterior to the eighth are often indistinguishable in adult Coleoptera. As a result, there arises the question as to whether a tergite or sternite takes part in the formation of the external genitalia. Muir and Singh Pruthi are agreed as to the improbability of this view and the results of other workers (e.g. Crampton, Christophers, Walker) are in accordance. Hopkins (24), however, considers that the spiculum gastrale represents the modified ninth sternite.

(2) The Efferent System.

According to Nussbaum (40, *Pediculus*, *Goniocotes*, and *Blatta*), the current impression that the efferent ducts of the larva unite to form the whole system of sexual ducts is incorrect; they form only the vasa deferentia, all other parts of the efferent system, viz. ejaculatory ducts and accessory glands, developing from the ectoderm. Christophers (*Diptera* and *Cimex*) regards the vasa deferentia as mesodermal structures and compares their mode of origin with that described by Zander in the Hymenoptera. Here he is at fault, since Zander evidently considers that the vasa deferentia arise

from the ectoderm. Verson and Bisson (*Bombyx mori*, 54) ascribe a mesodermal origin to the vasa deferentia and glands, while the ejaculatory duct develops as an ectodermal outgrowth. Wheeler (*Xiphidium*) states that except for a very short terminal posterior region which is ectodermal in origin, the whole of the efferent system is derived from the mesoderm. George is of the same opinion. Muir (*Coleoptera*) and Singh Pruthi (*Homoptera* and *Coleoptera*) find that the whole of the efferent system, even to the terminal portions of the vasa deferentia, is ectodermal in origin. Michaelis (*Honey-bee*), Seurat (*Doryctes gallicus*), and Kulagin (*Platygaster* sp.) also agree that all parts of the reproductive system, with the exception of the testes, are ectodermal in origin.

Again, Nussbaum, Verson and Bisson, and Michaelis are of the opinion that the ejaculatory duct arises from paired rudiments which subsequently fuse to form a median duct. Wheeler believes that all except the posterior terminal region is paired in origin, while Muir, Singh Pruthi, Christophers, Zander, Seurat, Kulagin, and George all state that the ejaculatory duct is unpaired in origin.

With reference to the accessory glands, the most generally accepted view is that of Escherich (19), viz. that one pair of glands is of mesodermal origin (the mesadenia), the other of ectodermal origin (the ectadenia). Escherich's work refers mainly to the *Coleoptera* and is confirmed by Blatter (3), while Bordas (4-6) is of the opinion that where one or more pairs of glands exist, they are of mesodermal origin. Recent work by George shows the same conclusion. Singh Pruthi, Nussbaum, and others regard all glands as having been derived from the ectoderm.

E. DEVELOPMENT.

(1) *Sitodrepa panicea* L.

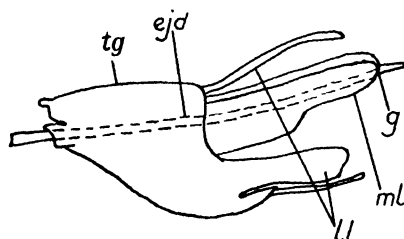
(a) Adult Structure.

Posterior to the eighth segment, the sternites are indistinguishable, a membranous area intervening between the eighth sternite and the anus. In repose, this area is folded into the body and forms the genital pocket within which the intromittent

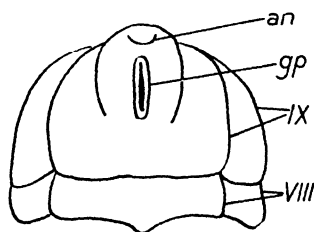
organ lies. The intromittent organ consists of a median lobe and two lateral lobes, which are asymmetrically placed, the median lobe piercing the left lateral lobe at its base (Text-fig. 3). The first connecting membrane is not present, the tegmen forming a ring at the base of the median and lateral lobes.

A heavily chitinized, horseshoe-shaped spiculum is present, lying against the ventral body-wall with its arms directed posteriorly: it gives attachment to powerful muscles.

The testes occupy a dorso-lateral position extending from the



TEXT-FIG. 3.



TEXT-FIG. 4.

Copulatory organ of *Sitodrepa panicea* L. $\times 100$. *ejd*, ejaculatory duct; *g*, gonopore; *ll*, lateral lobe; *ml*, median lobe; *tg*, tegmen.

Terminal abdominal segments of larva; ventral view. $\times 42$. *an*, anus of *S. panicea* L. *gp*, genital pocket; *IX*, Ninth segment; *VIII*, Eighth segment.

second to fourth abdominal segments. Each testis is composed of six testicular follicles. The vasa deferentia are short and in the fifth segment open into the lateral or paired branches of the ejaculatory duct. At the junction of the paired ejaculatory ducts are situated the openings of two pairs of accessory glands, the larger pair being internal, the smaller pair external. The median ejaculatory duct formed by the union of the paired ejaculatory ducts is short and straight. Posteriorly it pierces the genital pocket, traverses the tegmen, and opens at the apex of the median lobe.

(b) Structure of the Immature Insect.

(i) The External Organs. The Larva. In the larva, nine distinct tergites and sternites are present. The sternites

are much shorter than the tergites, with the result that normally the body is curled up with the head almost touching the tip of the abdomen. The ninth tergite is broad, and curves downwards and outwards so that from the ventral surface it appears somewhat horseshoe-shaped. Posterior to the ninth sternite, but not separated from it by a distinct suture, is a sclerite which fits into the concavity of the tergite. This sclerite may represent the whole, or a part of the tenth segment. Distally, it bears a papilla which protects the anus (Text-fig. 4).

Immediately anterior to the anus is a longitudinal depression which represents the primary invagination of the genital system. No genital plates or appendages are present in any of the larval instars, and it is, therefore, difficult to distinguish between the sexes.

The Pre-pupa. When the pre-pupal stage has been reached the male may be recognized by its smaller size.

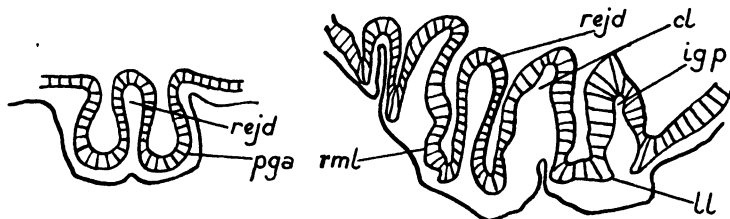
During this instar, besides the numerous changes involving the eversion of the appendages of the head and thorax, a certain amount of development of the external genitalia takes place. The depression posterior to the ninth sternite deepens, and in sections is seen to be an invagination of the ectodermal layer; this is the rudiment of the ejaculatory duct. On each side of the invagination, an appendage arises as an evagination of the ectodermal layer. Thus the gonopore is primarily bordered by a pair of appendages (Text-fig. 5).

This pair of primary appendages becomes doubled by the appearance of a vertical cleft (Text-fig. 6), and almost immediately the inner pair of appendages so formed fuses along its dorsal and ventral margins so that a tubular organ is formed which is pierced basally by the ejaculatory duct. The gonopore hence comes to lie at the apex of this organ which is the median lobe. At its base, the median lobe is not completely separated from the left lateral lobe, so that a certain amount of asymmetry occurs.

The Pupa. When pupation takes place, there is no reduction in the number of body segments; nine tergites and sternites can still be recognized. With the straightening of the abdomen, the anus moves caudad. Anterior to it are the three genital

appendages, the median lobe being the most prominent, and the left lateral lobe the smallest (Text-fig. 7).

With the maturation of the pupa a second invagination occurs

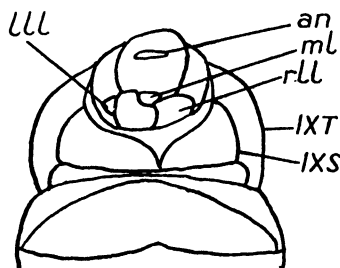


TEXT-FIG. 5.

TEXT-FIG. 6.

Longitudinal section through posterior border of ninth sternite in pre-pupa of *Sitodrepa panicea* L. *pga*, primary genital appendage; *rejd*, rudiment of ejaculatory duct.

Longitudinal section through posterior border of ninth sternite in older pre-pupa of *S. panicea* L. *cl*, cleft dividing median and lateral lobes; *igp*, invagination of genital pocket; *ll*, lateral lobe; *rml*, rudiment of median lobe; *rejd*, rudiment of ejaculatory duct.



TEXT-FIG. 7.

Terminal abdominal segments of pupa of *S. panicea* L. Ventral view. $\times 90$. *an*, anus; *lll*, left lateral lobe; *ml*, median lobe; *rl*, right lateral lobe; *IX T*, Ninth Tergite; *IX S*, Ninth Sternite.

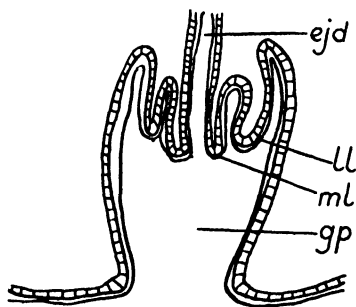
immediately anterior and ventral to the first; this is the genital pocket. As the process of invagination proceeds, the genital appendages become drawn in with the dorsal wall of the pocket. At the point of origin of the genital appendages, the ectodermal layer of the appendage is continuous with that of the pocket (Text-fig. 8).

In transverse sections, therefore, the lobes appear as diverti-

cula of the dorsal wall of the genital pocket, projecting downwards into its cavity (fig. 3, Pl. 7).

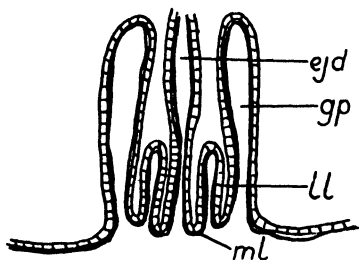
At a later stage of development the evagination of the apex of the pocket commences, carrying with it the genital appendages (Text-fig. 9).

Thus is formed a double-walled structure, the genital pocket,



TEXT-FIG. 8.

Longitudinal section showing invagination of genital pocket of *Sitodrepa panicea* L. *ejd*, ejaculatory duct; *gp*, genital pocket; *ll*, lateral lobe; *ml*, median lobe.



TEXT-FIG. 9.

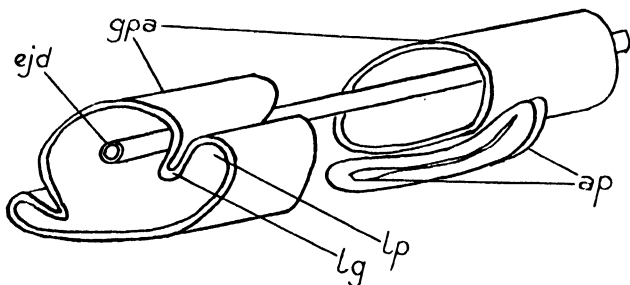
Longitudinal section showing evagination of genital pocket of *S. panicea* L. *ejd*, ejaculatory duct; *gp*, genital pocket; *ll*, lateral lobe; *ml*, median lobe.

pierced by the ejaculatory duct. The evagination proceeds until the genital lobes lie immediately within the mouth of the original invagination of the pocket as shown in Text-fig. 9. A transverse section through this region is illustrated in fig. 4, Pl. 7, which shows the outer wall of the genital pocket, the lateral lobes, and the median lobe pierced by the ejaculatory duct.

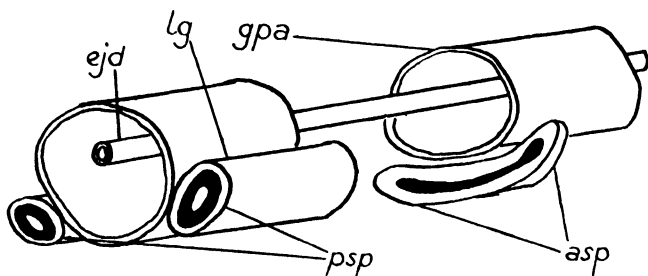
The spiculum gastrale arises in the following manner. During the early pupal stadium, before the process of evagination has taken place, a pair of lateral grooves is formed in the wall of the genital pocket. These grooves first make their appearance in the distal region of the pocket. Anteriorly they become very deeply sunken and a ventral blind pouch is wholly separated from the pocket (Text-fig. 10).

As the pupa matures the latero-ventral pouches formed by

the in-sinking of the grooves in the posterior region become completely constricted away from the wall of the pocket to form the two distally directed arms of the spiculum. These arms are connected anteriorly by the ventral pouch separated



TEXT-FIG. 10



TEXT-FIG. 11.

Stereogram of development of spiculum gastrale in *Sitodrepa panicea* L. *ap*, anterior ventral pouch; *ejd*, ejaculatory duct; *gpa*, outer wall of genital pocket; *lg*, lateral groove; *lp*, lateral pouch. Stereogram of development of spiculum gastrale in *S. panicea* L. *asp*, anterior convexity of spicule; *ejd*, ejaculatory duct; *gpa*, outer wall of genital pocket; *lg*, lateral groove; *psp*, posterior arm of spicule.

early in development. By the heavy deposition of chitin the spiculum eventually assumes the horseshoe formation familiar in the adult (Text-fig. 11).

(ii) The Efferent System. The Larva. In the larva, the only portions of the efferent system which are developed are the testes. These occupy a dorso-lateral position in the

second and third abdominal segments. Each is composed of six testicular follicles, the epithelial cells of which are rounded and closely packed, with dense contents and well-marked nuclei. Each follicle is invested by a sheath of connective tissue.

The Pupa. After pupation the testes are seen to extend from the second to fourth segments, growth having taken place in such a manner that the posterior region of each testis curves downwards to occupy a ventro-lateral position. The epithelial mass appears to have been subjected to a process analogous to segmentation, for, instead of forming a dense central core in each follicle, six or seven masses can be seen within the sheath of connective tissue (figs. 1 and 5, Pl. 7).

Towards its posterior distal region each follicle narrows considerably and passes into a short duct, the vas efferens, which is of smaller diameter than the follicle. Histologically these ducts consist of a solid core of epithelial cells invested by connective tissue, both layers of cells being continuous with the corresponding layers in the follicle (figs. 6 and 7, Pl. 7).

The six vasa efferentia of each side unite to form the vasa deferentia, which extend into the fifth abdominal segment, where they end blindly. As yet they have very small lumina (fig. 7, Pl. 7, Text-fig. 12).

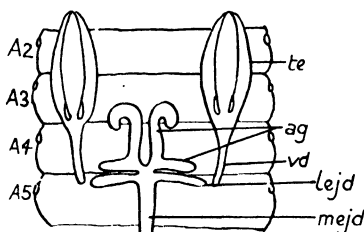
As the pupa matures, the vasa deferentia elongate and extend as far as, but not beyond, the posterior border of the fifth sternite (Text-fig. 13). They now possess distinct lumina. Near the testes, the vasa efferentia are still solid, but distally, before opening into the vasa deferentia, the cells of the epithelial layer become arranged around a central cavity. The vasa deferentia are of a similar structure to the vasa efferentia, but are of a greater diameter.

The ejaculatory duct, as already noted, arises from an ectodermal invagination situated posterior to the ninth sternite (Text-fig. 5). This invagination extends into the fifth segment, at the anterior border of which it divides into two short lateral branches. At its origin, each of these branches gives rise to an anteriorly directed blunt outgrowth, the rudiment of the median accessory gland.

The lateral divisions of the ejaculatory duct early become

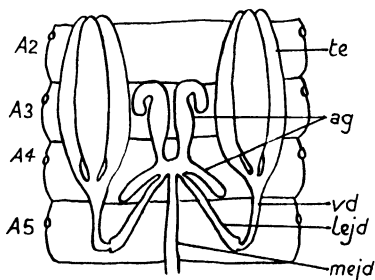
constricted longitudinally and give rise to two pairs of structures, the more posterior and ventral being the rudiments of the paired or lateral ejaculatory ducts, the more anterior and dorsal, the rudiments of the lateral accessory glands (fig. 2, Pl. 7). In Text-fig. 12, for the sake of clearness, the lateral accessory glands are shown as originating wholly anterior to the paired ejaculatory ducts; fig. 2, Pl. 7, shows more precisely the dorso-ventral arrangement.

As growth proceeds, the median ejaculatory duct elongates,



TEXT-FIG. 12.

Efferent system in young pupa of *Sitodrepa panicea* L. *ag*, accessory gland; *A 2-A 5*, second to fifth abdominal segments; *lejd*, lateral ejaculatory duct; *mejd*, median ejaculatory duct; *te*, testis; *vd*, vas deferens.



TEXT-FIG. 13.

Efferent system in mature pupa of *S. panicea* L. *ag*, accessory gland; *A 2-A 5*, second to fifth abdominal segments; *lejd*, lateral ejaculatory duct; *mejd*, median ejaculatory duct; *te*, testis; *vd*, vas deferens.

with the result that the point of origin of the paired ejaculatory ducts and the accessory glands is carried forwards into the fourth segment. The median accessory glands extend as far as the anterior border of the third segment. The paired ejaculatory ducts have also elongated, and now extend almost to the posterior border of the fifth segment, where they receive the openings of the mesodermal vasa deferentia (Text-fig. 13).

Histologically, the ejaculatory duct is composed of a layer of epithelial cells bounded by a basement membrane and a thick coat of muscular fibres. In a young pupa, the chitinous lining which is secreted by the epithelial cells and is characteristic of the mature pupa and adult has not yet been laid down (fig. 9,

Pl. 7). The glands are of a similar structure to the ejaculatory duct, but the muscular layer is poorly developed, while the epithelial cells are larger. The inner pair of glands is of a greater diameter than the ejaculatory duct and has a conspicuous lining of chitin (fig. 8, Pl. 7). The outer pair of glands is of a much smaller calibre.

(2) *Gastroidea polygoni* L.

(a) Adult Structure.

The testes lie one on either side of the alimentary canal, in the dorsal region, extending from the first to the fifth abdominal segments. Each testis is bilobed, the one lobe being slightly more anterior and dorsal, the other more posterior and lateral. A slender vas efferens arises from each lobe of the testis, the two ducts uniting in the third abdominal segment to form the vas deferens.

In the fourth segment, the vasa deferentia open into the lateral ejaculatory ducts. At their junction are situated a vesicula seminalis and a slender tubular accessory gland. The paired ejaculatory ducts unite in the fifth segment to form a common duct which runs forward to the anterior border of the fourth segment, here turning upon itself to pass into a much wider and dilated portion which extends to the anterior border of the seventh segment. The duct then narrows suddenly and bends forward again, running as far as the fifth segment almost to the point of union of the vasa deferentia. Here the duct makes another and final loop, and enters the copulatory apparatus at its anterior extremity as the ejaculatory duct proper. The gonopore is situated at the apex of a single median appendage.

The tegmen is ring-shaped, and situated in the outer wall of the genital pocket; both first and second connecting membranes are therefore present (Text-fig. 14).

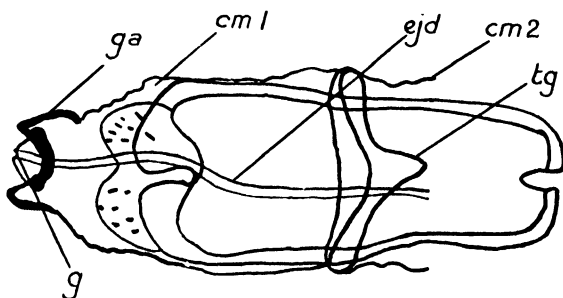
The spiculum is V-shaped and is closely attached to the genital pocket.

(b) Structure of the Immature Insect.

(i) The External Organs. The Larva. In the larva, nine abdominal tergites and sternites are visible. The anus is

borne by a sclerite situated between the ninth tergite and sternite and hence representing some portion of the tenth segment. No genital appendages are present, and the invagination of the genital pocket has not yet been commenced.

The Pre-pupa. During the pre-pupal instar various changes involving the development of the reproductive system occur. Posterior to the ninth segment, the genital pocket makes



TEXT-FIG. 14.

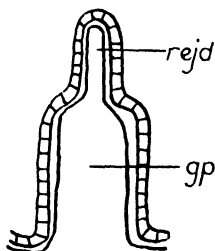
Copulatory organ of *Gastroidea polygona* L. $\times 55$. *cm 1*, 1st connecting membrane; *cm 2*, 2nd connecting membrane; *ejd*, ejaculatory duct; *g*, gonopore; *ga*, genital appendage; *tg*, tegmen.

its appearance as a wide-mouthed invagination of the ectodermis. This invagination extends anteriorly to the middle of the sixth segment, where it becomes considerably constricted and so continued within the body as the short and wide ejaculatory duct (Text-fig. 15).

As development proceeds, the proximal region of the genital pocket is evaginated, carrying with it the ejaculatory duct, the opening of which lies at the apex of the evagination. Thus the genital pocket forms a double-walled tube which is traversed by the ejaculatory duct, with the gonopore situated at its posterior extremity (Text-fig. 16).

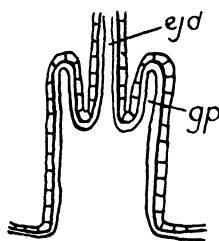
The genital appendages develop in the following manner. In the ectodermal layer at the apex of the genital pocket, four clefts make their appearance (fig. 11, Pl. 7). These clefts extend until the ejaculatory duct is completely separated from the wall of the genital pocket. At the same time, the cavity of

the ejaculatory duct is enlarged until it meets the dorsal and ventral clefts, aiding in the formation of a pair of plates bordering the gonopore (fig. 12, Pl. 7). The ectodermal layer of the inner margins of these plates is thus continuous with the epidermis lining the ejaculatory duct. The plates are simple appendages devoid of apical styli. At this stage of development, all the structures derived from the epidermis are thick-walled with greatly reduced lumina. The cavity of the genital pocket is reduced to a very small space surrounding the ejaculatory



TEXT-FIG. 15.

Longitudinal section through posterior border of ninth sternite in pre-pupa of *Gastroides polygona* L. *gp*, genital pocket; *rejd*, rudiment of ejaculatory duct.



TEXT-FIG. 16.

Longitudinal section through genital pocket in *G. polygona* L. immediately prior to pupation. *ejd*, ejaculatory duct; *gp*, genital pocket.

duct and genital appendages. No secretion of chitin has yet taken place within the genital pocket.

The Pupa. After pupation has taken place, nine tergites and sternites can still be identified. The sclerite representing the tenth segment bears, anterior to the anus, the longitudinal depression marking the opening of the genital pocket. No appendages are visible externally (Text-fig. 17).

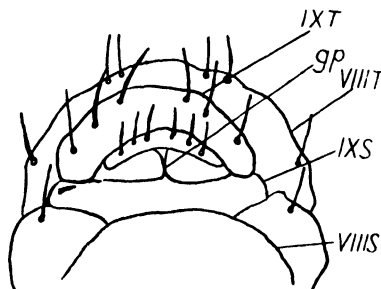
Further evagination of the genital pocket takes place, the apex now lying in the middle of the seventh segment. At the same time the ejaculatory duct elongates considerably, growth taking place in the posterior region.

The genital plates now become fused at their bases, the distal borders remaining free.

The spiculum gastrale is formed in a manner similar to that

already described for *Sitodrepa panicea* L., and Text-figs. 10 and 11 will again serve as illustrations. Posteriorly, between the seventh and eighth segments, a pair of latero-ventral folds arises in the genital pocket formed by the ingrowth of the wall. Anteriorly these folds converge, finally meeting and cutting off a ventral pouch. This pouch represents the anterior concavity of the spiculum, the posterior folds, its forks.

The maturation of the pupa and its transformation into the adult now consist of the elongation of the ejaculatory duct and its acquisition of a muscular layer; and in the considerable



TEXT-FIG. 17.

Terminal abdominal segments of pupa of *Gastroidea polygoni* L. Ventral view. $\times 35$. *gp*, genital pocket; *IX T*, ninth tergite; *IX S*, ninth sternite; *VIII T*, eighth tergite; *VIII S*, eighth sternite.

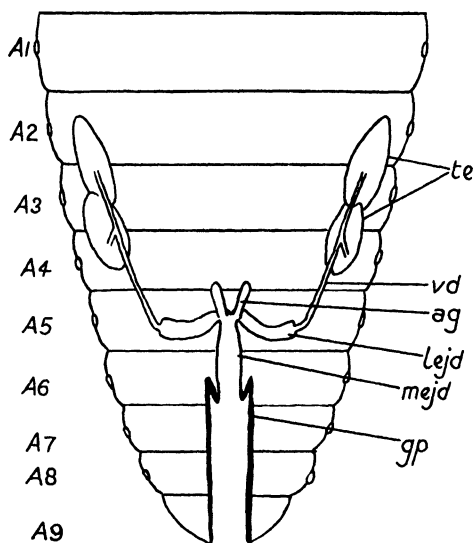
shrinkage of the walls of all organs of ectodermal origin, consequent upon the deposition of a thick layer of chitin by their cells.

(ii) The Efferent System. The Pre-pupa. No signs of the efferent system can be detected until the pre-pupal instar has been reached. In the pre-pupa, the testes occupy a dorsal position in the abdomen, extending from the middle of the second to the fourth segment. A vas efferens leaves each testicular lobe, uniting with its fellow of the same side to form the vas deferens. The vasa deferentia are slender ducts extending from the posterior border to the third segment to the middle of the fifth segment (fig. 10, Pl. 7, Text-fig. 18).

At this stage of development the ejaculatory duct is a short wide tube diverging in the middle of the fifth segment into two lateral or paired ejaculatory ducts, which form a loop to the

posterior border of the segment before bending forwards to unite with the vasa deferentia (Text-fig. 18). Arising at the point of division of the ejaculatory duct, and internal to the paired ducts, are two short glands (fig. 18, Pl. 8; Text-fig. 18).

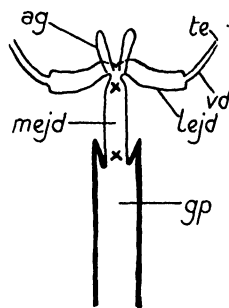
The Pupa. As growth proceeds, the testes enlarge considerably and extend, in the young pupa, to the anterior border



TEXT-FIG. 18.

Schematic representation of efferent system in pre-pupa of *Gas-troidea polygoni* L. *ag*, accessory gland; *A 1-A 9*, first to ninth abdominal segments; *gp*, genital pocket; *lejd*, lateral ejaculatory duct; *mejd*, median ejaculatory duct; *te*, testis; *vd*, vas deferens.

Growth points in efferent system. *x* marks the growth centres. Other lettering as above.

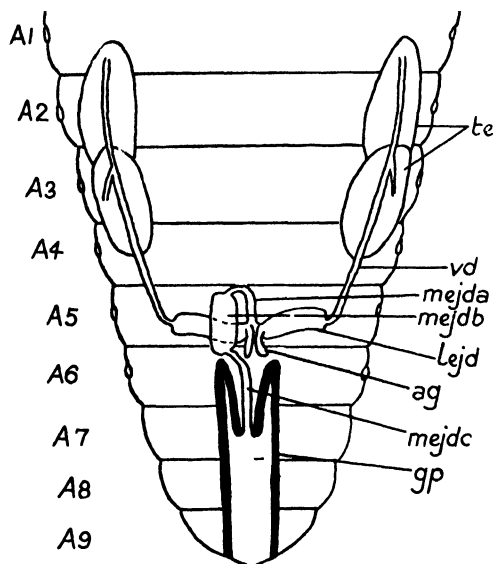


TEXT-FIG. 19.

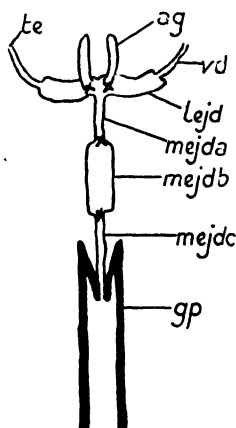
of the first abdominal segment. In old pupae they extend posteriorly into the fifth segment. The ejaculatory ducts are also subjected to elongation, and four growth-centres are involved, one posterior, one primary anterior, and a pair of secondary anterior centres.

The posterior growth-centre is a ring of tissue situated in the ejaculatory duct immediately anterior to, and surrounding, the

gonopore. The cells of the ectodermal layer at this point multiply rapidly, so that the original wide, thick-walled ejaculatory duct is gradually pushed anteriorly, and eventually out of the copulatory apparatus. The portion of the ejaculatory duct intercalated between the original invagination and the gonopore



TEXT-FIG. 20.



TEXT-FIG. 21.

Schematic representation of efferent system in young pupa of *Gasteroidea polygoni* L. *ag*, accessory gland; *A 1-A 9*, first to ninth abdominal segments; *gp*, genital pocket; *lej*, lateral ejaculatory duct; *mej*, median ejaculatory duct, (*a*) anterior region, (*b*) median region, (*c*) posterior region; *te*, testis; *vd*, vas deferens.

Growth points in efferent system. *x* marks the growth centres. Other lettering as above.

is a long tube, slender and with a very fine calibre (figs. 16 and 21, Pl. 8). When growth in this region is complete, this slender duct emerges from the copulatory apparatus in the fifth segment and here makes a loop backward (fig. 15, Pl. 8) to the anterior border of the seventh segment. The duct now bends forwards again (fig. 16, Pl. 8) and passes into the original wide tube.

This condition is found towards the end of the pupal instar (Text-fig. 22).

At the same time growth is taking place in the anterior region. The primary anterior growth-centre is situated immediately posterior to the division of the ejaculatory duct. Growth in this region results in the intercalation of a second slender duct between the bases of the paired ejaculatory ducts and the proximal extremity of the original invagination. It proceeds in such a manner that the point of union between the paired ejaculatory ducts and the vasa deferentia remains in the fifth segment. The intercalated section is thrust forwards in a loop, reaching to the posterior border of the third segment, where it passes into the original tube (fig. 13, Pl. 7; Text-figs. 19-23).

In the mature pupa, then, the unpaired portion of the ejaculatory duct is divisible into three regions (Text-figs. 22 and 23).

(1) The slender ejaculatory duct proper which traverses the copulatory apparatus and opens at the gonopore (fig. 16, Pl. 8), leaving the copulatory apparatus in the fifth segment and thence running posteriorly again into the sixth to pass into

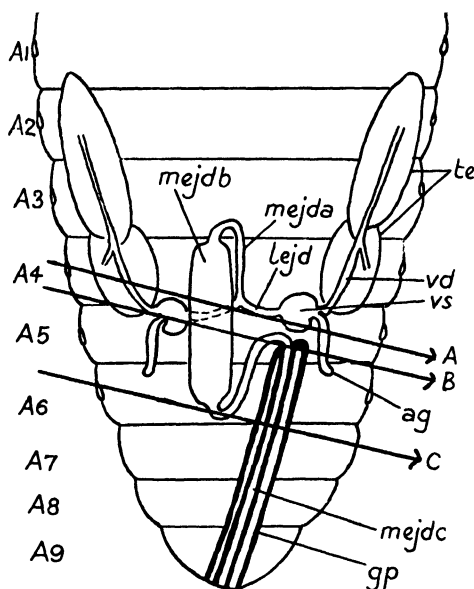
(2) the original wide invagination which has the form of a sac extending from the middle of the sixth to the posterior border of the third segment (fig. 13, Pl. 7; figs. 14 and 15, Pl. 8). Here it passes into

(3) the anterior slender duct (fig. 13, Pl. 7) which now runs posteriorly to the fifth segment, where it divides to form the paired ejaculatory ducts.

The two secondary anterior growth-centres are to be found, one at the base of each lateral ejaculatory duct (Text-fig. 21).

Growth here results in the lateral extension of the ducts in the fifth segment, so that their points of union with the vasa deferentia are removed farther from the mid-ventral line (figs. 14 and 15, Pl. 8; Text-fig. 22). At the growth points, each ejaculatory duct becomes swollen considerably to form the vesicula seminalis at the base of the gland. The latter is seen in sections to be slightly sunken into the cavity of the swelling (fig. 14, Pl. 8; Text-figs. 22 and 23).

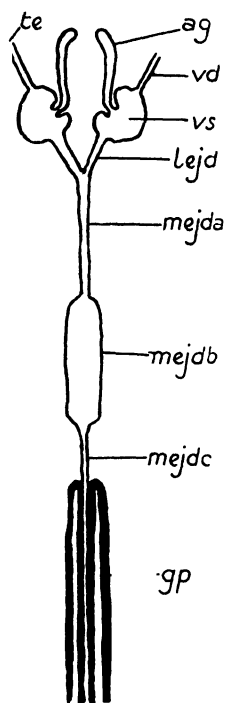
Histologically the vasa deferentia are seen to be of mesodermal origin. They are slender ducts composed of a single layer of small cells directly continuous with the epithelial layer of the



TEXT-FIG. 22.

Schematic representation of efferent system in mature pupa of *Gastroides polygoni* L. *ag*, accessory gland; *A 1-A 9*, first to ninth abdominal segments; *gp*, genital pocket; *lejd*, lateral ejaculatory duct; *mejd*, median ejaculatory duct, (*a*) anterior region, (*b*) median region, (*c*) posterior region; *te*, testis; *vd*, vas deferens; *vs*, vesicula seminalis.

Growth points in the efferent system. Lettering as above.



TEXT-FIG. 23.

testis. They are invested by a delicate sheath of connective tissue (fig. 17, Pl. 8). The ejaculatory duct, glands, and vesiculae seminales are ectodermal in origin. During the pre-pupal instar their walls are composed of a single layer of large cells, bounded externally by a basement membrane. No secretion lines the lumina. In the mature pupa the terminal portion of

the ejaculatory duct is extremely slender. The cells lining it are very small, the muscular layer is poorly developed, and the cavity of the duct is very small indeed (fig. 21, Pl. 8). The middle, wide portion of the duct is lined with large cells and has a prominent muscular coat (fig. 20, Pl. 8). Its lumen is conspicuous and is lined with chitin. The anterior regions of the ejaculatory duct, glands, and vesiculae seminales are of a similar structure. The glands, vesiculae seminales, and posterior region of the ejaculatory duct have no muscular investments (Glands, figs. 18 and 19, Pl. 8).

It is possible to distinguish the vas deferens from the lateral branches of the ejaculatory duct at any stage of development owing to the differences in size and histology. The disparity in calibre is most noticeable in the pre-pupal instar, while the presence or absence of the chitinous lining is a criterion in the more mature insect.

(3) *Anthonomus pomorum* L.

(a) Adult Structure.

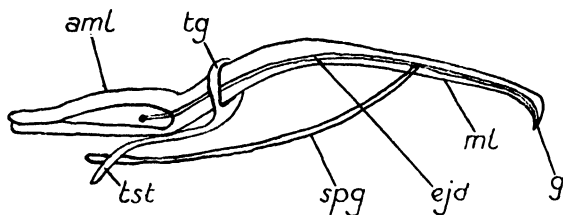
The testes are bi-lobed, the one lobe lying posterior to the other. From the testes the vasa deferentia lead posteriorly to open into the paired ejaculatory ducts. Posterior to their junction with the vasa deferentia, the paired ejaculatory ducts are dilated to form vesiculae seminales, the openings of a pair of accessory glands being situated immediately anterior to the latter. The median ejaculatory duct is formed by the union of the paired ejaculatory ducts and is a fine tube piercing the copulatory apparatus.

Both connecting membranes are present, the tegmen is ring-shaped with an anteriorly directed strut, while the median lobe is slender and curved, forking anteriorly. The spiculum gastrale is a simple, straight rod (Text-fig. 24).

(b) Structure of the Immature Insect.

(i) External Organs. The Larva. Nine tergites and sternites are present, the anus being situated on a sclerite posterior to the ninth sternite. No genital appendages are visible externally. Transverse sections of a fully grown larva,

about to enter the quiescent pre-pupal instar, show that the genital pocket has already developed as a shallow ectodermal invagination immediately posterior to the ninth sternite. The cells lining the invagination are large and closely packed, and the ventral wall gives rise to a pair of thickenings which are



TEXT-FIG. 24.

Copulatory organ of *Anthonomus pomorum* L. $\times 50$. *aml*, anterior fork of median lobe; *ejd*, ejaculatory duct; *g*, gonopore; *ml*, median lobe; *spg*, spiculum gastrale; *tg*, tegmen; *tst*, tegmental strut.

the rudimentary genital appendages. Between the appendages the genital pocket is prolonged into the body-cavity as a very short blind tube, the rudiment of the ejaculatory duct (Text-fig. 25).

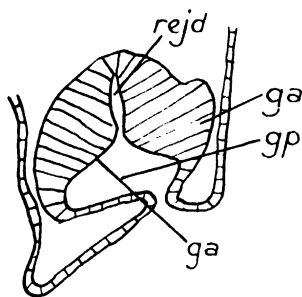
The Pupa. After pupation has taken place the abdomen is still seen to consist of nine tergites and sternites, the ninth tergite bearing a pair of stout caudal spines. Immediately posterior to the ninth sternite is situated the mouth of the genital pocket (Text-fig. 26).

In transverse sections it can be seen that further invagination of the genital pocket has taken place and that this now extends as far as the posterior border of the sixth segment. A certain amount of evagination of its proximal region has already taken place, and the gonopore, bordered by the basally fused appendages, lies at the posterior border of the seventh segment (fig. 23, Pl. 8). As the pupa matures, growth of the genital pocket is continued until it reaches the middle of the fifth segment. At the same time further evagination takes place and eventually the gonopore lies at the apex of the genital pocket.

The spiculum gastrale originates during the pupal instar as a ventral fold of the genital pocket extending from the fifth to the seventh segments. The fold becomes entirely constricted

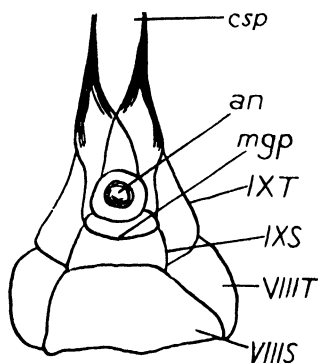
from the pocket and lies below it as an ectodermal tube (fig. 26, Pl. 8). The deposition of chitin gives it the familiar rod-like appearance. Text-fig. 27, A, B, and C, illustrate stages in its development.

Before the process of chitinization is complete, the abdominal segments posterior to the seventh become withdrawn into the



TEXT-FIG. 25.

Longitudinal section through posterior border of ninth sternite of *Anthrenus pomorum* L. *ga*, genital appendage; *gp*, genital pocket; *rejd*, rudiment of ejaculatory duct.



TEXT-FIG. 26.

Terminal abdominal segments of pupa of *A. pomorum* L. Ventral view. $\times 60$. *an*, anus; *csp*, caudal spine; *mgs*, mouth of genital spine; *IX T*, ninth tergite; *IX S*, ninth sternite; *VIII T*, eighth tergite; *VIII S*, eighth sternite.

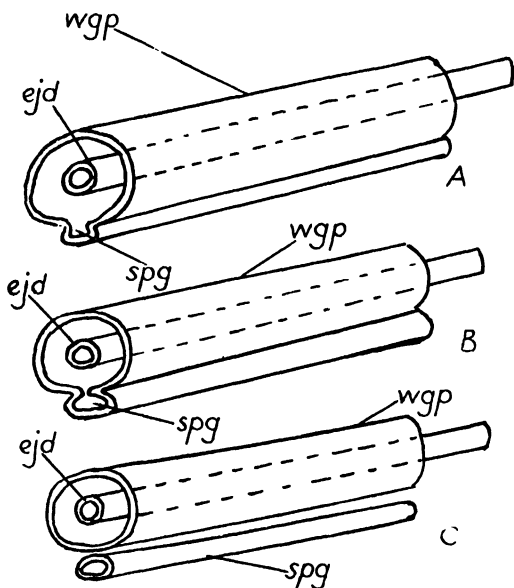
body-cavity, so that in the adult beetle only seven abdominal segments can be recognized.

The deposition of chitin in the walls of the genital pocket now takes place, differentiating connecting membranes, tegmen, median lobe, and spiculum gastrale. With the secretion of chitin the cells of the ectodermal layer decrease noticeably in size.

(ii) The Efferent System. In the young pupa the testes are very little developed. The anterior lobe is situated in the fourth, the posterior lobe in the fifth segment. A fine duct leaves each lobe at its hinder border, that of the anterior extending to the posterior border of the fourth segment, that of the posterior being very short. The ducts are very fine indeed and

at this stage of development have no communication with each other (Text-fig. 28).

The ejaculatory duct is now a thick-walled, wide tube extending to the posterior border of the sixth segment. Here it divides into two lateral branches. Each of the latter shortly

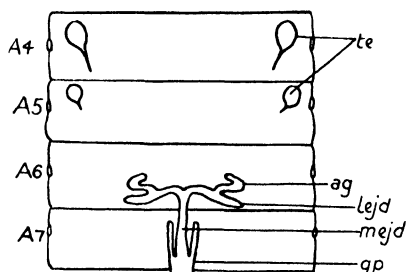


TEXT-FIG. 27, A, B, C.

Stereogram of development of spiculum gastrale in *Anthrenus pomorum* L. *ejd*, ejaculatory duct; *spg*, spiculum gastrale; *wgp*, wall of genital pocket.

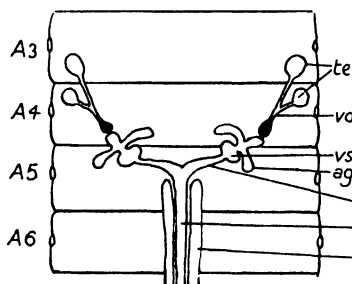
re-divides in the dorso-ventral plane, the dorsal division being the rudiment of the pair of accessory glands, the ventral of the lateral or paired ejaculatory ducts (fig. 22, Pl. 8; Text-fig. 28). The glands and ducts are thick-walled blind structures with wide cavities.

As the pupa matures and the genital pocket lengthens, the point of divergence of the ejaculatory ducts becomes carried forwards into the fifth segment. The ducts themselves lengthen and extend into the fourth segment. The rudiment of the gland becomes longitudinally divided into two and immediately

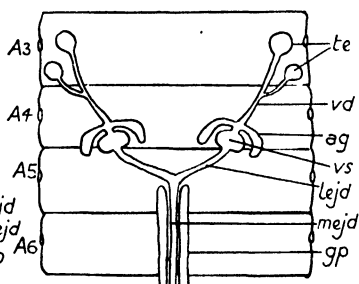


TEXT-FIG. 28.

Schematic representation of efferent system in young pupa of *Anthonomus pomorum* L. *ag*, accessory gland; *A 4-A 7*, fourth to seventh abdominal segments; *gp*, genital pocket; *lejd*, lateral ejaculatory duct; *mejd*, median ejaculatory duct; *te*, testis; *vd*, vas deferens.



TEXT-FIG. 29.



TEXT-FIG. 30.

Schematic representation of efferent system in maturing pupa of *Anthonomus pomorum* L. *ag*, accessory gland; *A 3-A 6*, third to sixth abdominal segments; *gp*, genital pocket; *lejd*, lateral ejaculatory duct; *mejd*, median ejaculatory duct; *te*, testis; *vd*, vas deferens; *vs*, vesicula seminalis.

Schematic representation of efferent system in an old pupa of *A. pomorum* L. *ag*, accessory gland; *A 3-A 6*, third to sixth abdominal segments; *gp*, genital pocket; *lejd*, lateral ejaculatory duct; *mejd*, median ejaculatory duct; *te*, testis; *vd*, vas deferens; *vs*, vesicula seminalis.

posterior to the openings of the glands the lateral ejaculatory ducts are dilated to form the vesiculae seminales (figs. 24 and 25, Pl. 8).

The testes undergo a forward migration and now lie in the third abdominal segment. The ducts from the two lobes unite at the anterior border of the fourth segment, and the vas

deferens so formed is a slender tube provided with a distinct lumen. It is of very much smaller diameter than the derivatives of the ejaculatory duct and is easily distinguishable therefrom. Posteriorly the vas deferens becomes rather swollen and its lumen is obliterated (fig. 24, Pl. 8). The swollen ampulla thus formed is applied to the blind end of the ejaculatory duct (Text-fig. 29).

Eventually the intervening walls break down. The hitherto solid ampulla acquires a lumen and communication between the cavities of the vas deferens and ejaculatory duct is established (Text-fig. 30).

The ejaculatory duct and its derivatives, viz. vesiculae seminales and glands, have the thick-walled, chitin-lined structure characteristic of organs of ectodermal origin (figs. 29 and 30, Pl. 8), while the vasa deferentia are lined by much smaller cells and have no secreted intima (figs. 27 and 28, Pl. 8).

F. CONCLUSIONS.

From the foregoing studies the following conclusions may be reached.

(1) The External Organs.

(a) The Intromittent Organ.

The mode of development of the intromittent organ in the Coleoptera is similar to that in other orders of the Insects, viz. it develops from a pair of appendages of the ninth segment. In *Sitodrepa panicea* L. these appendages actually develop as diverticula of the posterior border of the ninth sternite and are externally visible throughout the pupal instar. In *Tenebrio molitor* L., *Gastroidea polygoni* L., and *Anthonomus pomorum* L. the appendages develop within the genital pocket from the ectodermal layer bordering the gonopore. Since the latter is situated immediately posterior to the ninth sternite, the appendages arise in this case also from the posterior border of that segment.

The intromittent organ in the adult may be either of the trilobe types, when a median lobe and a pair of lateral lobes are present as in *Sitodrepa panicea* and *Tenebrio*

molitor, or there may be a median lobe only as in *Gastroidea polygona* and *Anthonomus pomorum*.

In the former case, the median lobe arises by the fusion of a second pair of appendages which is cut off from the primary appendages; in the latter, the primary pair of appendages fuses to form the median lobe without any preliminary division.

From their median position, one on each side of the gonopore, their unsegmented nature and late appearance in development, it appears that the genital appendages in the Coleoptera are homologous with the parameres in other Insecta and hence correspond with the endopodites of the Crustacean limb.

The genital papillae which, according to Singh Pruthi, are present in the larvae of *Tenebrio molitor* and represent the coxites, were not observed in the types studied in the present paper or in the larvae of *Ceuthorrhynchus pleurostigma* and *Rhagium bifasciatum* which were examined.

In other orders of the Insecta where coxites are present, e.g. in the Homoptera, these appear at an early stage in development and are either separate from the parameres from the commencement as in the Homoptera, or become divided off from them at an early stage of development as in the Trichoptera and Hymenoptera. The parameres are differentiated at a much later stage of development.

George's insistence that the parameres in the Homoptera are nothing more than outgrowths of the aedeagus (20), emphasizes a very real need for the adoption of a system of nomenclature that shall be universally applicable. In the example cited, the distinction is of little significance as both parameres and aedeagus are known to have been derived from a pair of primary appendages corresponding with the endopodites or telopodites of the ninth segment. Hitherto, too much stress has been laid on the secondary division of the endopodites. The term 'paramere' in its original application refers to the endopodites of the ninth segment, and if a satisfactory name for the parameres and their derivatives can be adopted, an important step will have been made towards the simplification of the subject. It is therefore suggested that the term paramere be completely

rejected and that the following terms be applied to the derivatives of the endopodites of the ninth segment. (1) The aedeagus when only a median appendage or a single pair of appendages is present. (2) The median and lateral lobes of the aedeagus when a median and a pair of lateral appendages are present.

The terms selected are not new, but their adoption would serve to introduce order into the chaos which at present exists.

With regard to the question as to whether the tri-lobe form is more primitive than the single lobe form, Sharp and Muir consider the former the more primitive, the latter being derived therefrom.

There seems to be a close connexion between the loss of the first connecting membrane and the presence of the tri-lobe intromittent organ, while in the single lobed type both connecting membranes are present. Which of these represents the primitive condition, however, is still an unsettled question and cannot be satisfactorily decided as yet.

(b) The Spiculum gastrale.

In *Sitodrepa panicea* and *Gastroidea polygoni* the spiculum gastrale was seen to arise as a pair of lateral folds in the wall of the genital pocket which later became completely constricted therefrom; in *Anthonomus pomorum* it was derived from a ventral pouch cut off from the genital pocket. In *Tenebrio molitor* the spiculum was stated to have arisen as a pair of ectodermal invaginations of the body-wall, just posterior to the ninth segment. While the mode of origin in the three cases differs slightly, viz. in the first two being derived from an invagination of the body-wall, in the third directly from the body-wall, they may still be termed homologous structures.

A similar difference in mode of origin occurs in the genital appendages, which in *Sitodrepa panicea* arise from the body-wall and in *Tenebrio molitor*, *Gastroidea polygoni*, and *Anthonomus pomorum* are derived from the wall of the genital pocket.

(c) The Body Segments.

Since the spiculum gastrale and the genital pocket are already in existence at a time when the full complement of tergites and sternites is visible, it is impossible that a tergite or a sternite should form the whole, or a part of, the structures in question.

(2) The Efferent System.

(a) The vasa deferentia are derived from the original efferent passages and are hence mesodermal in origin, not extending beyond the posterior border of the seventh segment as is usual in the Insecta.

There are, however, conflicting accounts of the development of these ducts. Wheeler, for example, describes the embryonic rudiments of the vasa deferentia in *Xiphidium*, as extending into the ninth segment, as do Christophers in the Diptera and George in the Homoptera. The majority of other authors, e.g. Packard (41), Korschelt and Heider (29), Singh Pruthi, state definitely that the original efferent passage terminates in the seventh segment. Moreover, these rudiments, according to Wheeler, Christophers, Verson and Bisson, and George, are wholly mesodermal in origin, while Muir, Singh Pruthi, Packard, and Zander are of the opinion that at least the terminal portions of the vasa deferentia are derived otherwise than from the original mesodermal rudiments. The following extract from Packard, ex Korschelt and Heider, illustrates the latter view: 'In the male . . . it (i.e. the original efferent passage) is not along its whole length transformed into the vas deferens, but its terminal distal portion degenerates and is replaced by a newly formed terminal portion of the vas deferens, which unites with the ectodermal ductus ejaculatoris.'

From his study of the efferent ducts in *Tenebrio molitor* Singh Pruthi fails to find any trace of the vasa deferentia as mesodermal structures, and hence concludes that the degeneration of the original efferent duct has taken place to such an extent that the vasa deferentia are confined wholly to the region of the testes. He concludes, further, that the ducts which function as vasa deferentia in *Tenebrio molitor* are secondary structures of ectodermal origin, extending beyond

the posterior limit of the mesodermal vasa deferentia, viz. the posterior border of the seventh segment. In support of this statement, he refers to his own work on the Homoptera, in which the vasa deferentia or paired ejaculatory ducts, as he subsequently names them, are derived from ectodermal structures, terminating near, though not actually opening on, the eighth abdominal segment. He also quotes from Muir to the effect that: 'The zygos (vasa deferentia) are supposed to be of mesodermal origin, but they seem to develop continuously from the stenazygos (ejaculatory duct).'

In all the three species examined the vasa deferentia are present as slender ducts of mesodermal origin. In *Sitodrepa panicea* they extend to the posterior border of the fifth segment, in *Gastroidea polygoni* to the posterior border of the fourth, and in *Anthonomus pomorum* only to the middle of the fourth segment. A varying degree of degeneration of the terminal region of the original efferent passages has therefore taken place. This degeneration has been compensated for by the development of secondary structures, the lateral or paired ejaculatory ducts, which arise early in the pre-pupal instar as outgrowths of the median ejaculatory duct. They are hence ectodermal in origin and as a rule do not extend farther than the fifth abdominal segment. A statement to the effect that the paired ducts of the efferent system are wholly mesodermal, or wholly ectodermal in origin, must, therefore, be modified. The ducts appear to be partly mesodermal and derived from the original efferent passages, partly ectodermal in origin, the extent of each region varying with the species.

(b) With the exception of the vasa deferentia, the efferent system is ectodermal in origin. So far as the ejaculatory duct is concerned, this statement is now generally accepted as correct. With reference to the accessory glands, however, there is some difference of opinion.

According to Escherich, whose work refers mainly to the Coleoptera, the accessory glands in the male may be divided into two categories—those which arise as diverticula of the vas deferens and are hence mesodermal in origin (mesadenia), and those which arise as diverticula of the ejaculatory duct and

are hence ectodermal in origin (ectadenia). Blatter confirms this view, while Bordas states that the glands, whether one or more pairs are in existence, are of mesodermal origin. Christophers, Verson and Bisson, and George are also of the opinion that the glands are of mesodermal origin. Singh Pruthi notes that the views of Escherich and Blatter need modification, himself describing both pairs of glands as arising from diverticula of the (functional) vas deferens, and hence probably of ectodermal origin.

It would appear that the glands in *Sitodrepa panicea*, *Gastroidea polygona*, and *Anthonomus pomorum* all arise as diverticula of the lateral ejaculatory ducts. Histologically the glands closely resemble the ejaculatory duct. Furthermore, they are frequently so much larger in calibre that it is unlikely that they should have originated as outgrowths of the slender vasa deferentia.

In *Anthonomus pomorum* and *Gastroidea polygona* the vesiculæ seminales are obviously dilations of the paired ejaculatory ducts, and, like them, of ectodermal origin.

(c) That the efferent system, other than the vasa deferentia, is unpaired in origin.

Nussbaum, Michaelis, and Verson and Bisson seem to be the last advocates of the theory of the paired origin of the ejaculatory duct, Wheeler regarding the terminal ectodermal region as unpaired in origin.

In *Sitodrepa panicea*, *Gastroidea polygona*, and *Anthonomus pomorum*, there is no doubt that the ejaculatory duct is unpaired from the very beginning, hence its derivatives also, viz. the paired glands and vesiculæ seminales, must also be regarded as fundamentally unpaired in origin.

PART II. THE FEMALE.

A. GENERAL STRUCTURE AND NOMENCLATURE.

In general plan and arrangement, the reproductive organs of the adult Coleopteran female resemble those of the male. A pair of ovaries occupies a dorso-lateral position, extending through one or more of the abdominal segments between the

first and the seventh. Each ovary is subdivided into two or more ovarioles, a slender duct leading from each into the oviduct. The oviducts are paired and of varying length and calibre. They unite with the lateral branches of the uterus. These unite together to form a median duct which may be termed the uterus or the vagina. Opening into the mid-dorsal region of the uterus, a little posterior to the junction of the paired branches, is a blind sac. This sac receives the duct of the accessory gland and the opening of the tubular spermatheca.

The uterus opens posteriorly to the ninth segment. The gonopore is usually borne at the apex of a median tubular appendage and is bordered by a pair of palpi.

Posterior to the eighth segment the body-wall remains membranous, as in the male, and the segments are withdrawn into the body-cavity.

Ventral to the uterus is a Y-shaped rod which serves as the basis of attachment for the muscles which control the uterus and the associated structures. As in the male, therefore, the reproductive system may be considered under two headings:

- (1) The External Organs or Ovipositor.
- (2) The Efferent System, comprising ovaries and oviducts, uterus, spermatheca, and accessory glands.

B. HISTORY OF THE SUBJECT AND HOMOLOGIES.

(1) The Ovipositor.

The ovipositor in the Insecta generally is derived from three pairs of lobes which arise in the larval instar, one pair on the eighth and two pairs on the ninth sternite. This mode of development has been described by Zander in the Hymenoptera (58), Haviland in *Lygoceros* sp. (21), and Kraepelin in the Honey-bee (28). Seurat (46) describes the development of the first pair of lobes in *Doryctes gallicus* from the seventh segment, and of the other two pairs from the eighth. If, as seems likely, he has included the first abdominal segment with the thorax, these segments being normally fused together in the Hymenoptera-Apocrita, then the seventh and eighth segments here will correspond to the eighth and ninth segments in

other Insecta. Wheeler, in *Xiphidium* (57), states that the ovipositor lobes are developed from outgrowths of the eighth, ninth, and tenth segments, those of the tenth having migrated anteriorly to lie between those of the ninth during development. This view has received little support from the investigations of other workers.

As in the male, so in the female, Verhoeff (52) compares the three pairs of ovipositor lobes with the primitive genital appendages in *Machilis*. He gives the following table of comparison for the females of Pterygote insects:

Eighth abdominal segment. Two Gonocoxites or a Coxosternum. Two Telopodites (Anterior Ovipositors) or absent.

Ninth abdominal segment. Two Gonocoxites, or absent, but never a coxosternum. Two Telopodites (Posterior Ovipositors) or absent.

That is to say, that the anterior or ventral ovipositor lobes represent the telopodites or endopodites of the eighth segment; the dorsal ovipositor lobes, the telopodites or endopodites of the ninth segment; the posterior or lateral ovipositor lobes, the coxites of the ninth segment.

The most recent work on the subject of the homologies of these appendages, viz. that of Crampton (7-15), Walker (55), and Singh Pruthi (43 and 45), is entirely in agreement with this basic plan laid down by Verhoeff. Nel (39) considers that the anterior ovipositor lobes are homologous with the coxites and not the telopodites of the eighth segment.

Zander does not agree that the ovipositor lobes are the homologues of segmental appendages. The following quotations make clear his attitude to the subject: 'The earliest rudiments of the thoracic legs are found in *Apis* in the embryo, while the rudiments of the sexual appendages do not appear until the larva is four days old.'

Also: 'All these facts show clearly that the rudiments of the embryonic abdominal appendages and the gonapophyses in the Hymenoptera belong to two different periods of development; the former are of a true embryonic nature and are only present in the embryo; the latter first appear in the larval instar. And so long as the change of the one into the other has not been

observed, no author can prove that the gonapophyses and the embryonic abdominal appendages are the same structures.'

Nevertheless, it is generally accepted by the most recent investigators, that the ventral ovipositor lobes represent the endopodites of the eighth segment, the dorsal lobes the endopodites, the lateral lobes the coxites of the ninth. Furthermore, Zander's reasoning is faulty: the larva of *Apis* is apodous, yet it is not doubted that the embryonic thoracic appendages are the forerunners of those legs which are present in the pupa and adult, although wanting in the larva.

In the Coleoptera the gonopore is bordered by a pair of plates bearing palps at their apices. These plates have been variously described as the divided seventh, eighth, or ninth sternite. Verhoeff (50) originally believed that ten tergites and sternites were present in the female Coleoptera, and that the ovipositor was formed from the posterior segments of the body which are retractile. The plates bordering the gonopore represented the divided tenth sternite, and the palpi at their apices the modified cerci. Moreover, he asserted that the form of the ovipositor was modified by function, and that three main types of modification occurred, namely:

- (a) When the eggs were deposited in the ground, the ovipositor was provided with 'Grabinstrumenten'.
 - (b) When in splits and cracks in wood, with a 'Legerohre'.
 - (c) When in the tissues of young plants with a 'Legesabel'.
- If the eggs were merely deposited broadcast, then no ovipositor was developed.

Although Verhoeff modified this view considerably, and in later years considered the ovipositor to be of a true appendicular nature (52), there seems to linger a general belief, based on this former paper of his, that the distal abdominal segments in the Coleoptera have become telescoped one into another, to form a retractile tube which functions as an ovipositor (25).

The most recent work on the subject is by Singh Pruthi, who has studied the development of the genital plates in the larva and pupa, and considers them to be homologous with the lateral ovipositor lobes in other Insecta. Hence they represent the coxites of the ninth segment.

(2) The Efferent System.

While the gonopore in the male is constantly located posterior to the ninth sternite, there is considerable variation in position of the female aperture in the different orders.

In the Ephemeroptera (33) there is a pair of apertures, the oviducts opening separately posterior to the seventh sternite; in the Orthoptera, the gonopore is posterior to the seventh sternite according to Wheeler, to the eighth according to Walker and Crampton, to the seventh in such forms as *Blatella* and *Forficula*, and on the eighth in *Locustana* and *Colemania*, according to Nel (39); in the Hemiptera-Homoptera (Singh Pruthi and George, 20) and Heteroptera (Christophers and Cragg, 16) posterior to the eighth sternite; in the Diptera (Awati, 1; Christophers, 18) posterior to the eighth segment; in the Macrolepidoptera (Jackson, 26) there are two apertures, one posterior to the eighth sternite, the other posterior to the ninth; in *Bombyx mori* (Verson and Bisson, 54) one aperture posterior to the ninth; in the Hymenoptera (Kraepelin, 28; Haviland, 21; Kulagin, 30; and Zander, 58) and Coleoptera (Singh Pruthi) the gonopore is located posterior to the ninth sternite.

This variability raises the important question as to whether the gonopore in the different orders is strictly homologous. Singh Pruthi suggests that this is not so, but that three conditions are represented:

- (a) Where there is a pair of apertures posterior to the seventh sternite, these represent the openings of the oviducts;
- (b) Where there is a single aperture or gonopore posterior to the eighth sternite, this corresponds to the opening of the uterus;
- (c) Where there is a single aperture or gonopore posterior to the ninth sternite, this is the opening of the spermatheca.

George and Nel also suggest that the evolutionary tendency lies in the posterior shifting of the gonopore.

An interesting problem arises here which will be more fully discussed in Part III, namely, whether the uterus in the female is homologous with the ejaculatory duct in the male, and to

which, if any, of the three apertures in the female does the male gonopore correspond?

Much confusion is also associated with reference to the mode of development of the uterus and its appendages. Herold (22) and Balbiani (2) state that the uterus is formed by the fusion of the posterior regions of the paired oviducts and is hence mesodermal in origin; Vernon and Bisson, that the uterus and vagina are derived from paired ectodermal vesicles in the eighth and ninth segments which subsequently fuse to form a median duct; Nussbaum (40), that the efferent ducts arise as paired rudiments of ectodermal origin, the azygos condition being secondary and consequent upon the fusion of the zygos rudiments; Jackson, Seurat, Kulagin, Heymons (23), Christophers, Singh Pruthi, George and Nel, that the uterus is unpaired and ectodermal in origin.

Opinions being so much at variance, the development of the genital system in *Sitodrepa panicea* L., *Gastroidea polygoni* L., *Anthonomus pomorum* L., and *Rhagium bifasciatum* F. is of interest.

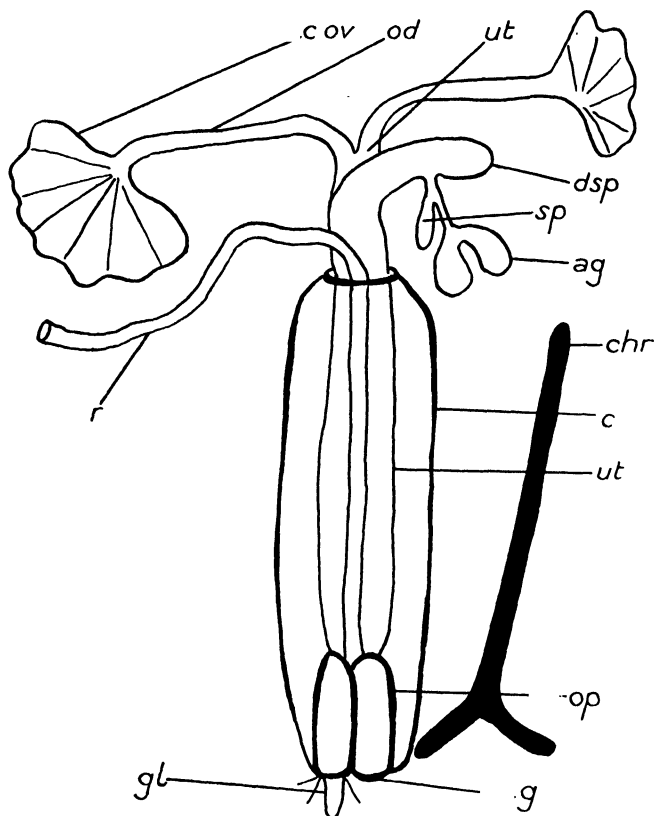
C. DEVELOPMENT.

(1) *Sitodrepa panicea* L.

(a) Adult Structure (Text-figs. 31 and 32).

The ovaries form large compact masses extending from the second to the fourth abdominal segments. The oviducts are short and run transversely towards the middle line where they unite to form the uterus. A short distance posterior to the junction of the two oviducts a dorsal pouch opens into the uterus. This dorsal pouch receives the common duct of a tubular spermatheca and the bi-lobed accessory gland. In the seventh segment, together with the rectum, the uterus enters a double-walled chitinous cylinder formed by the telescoping of the posterior abdominal segments into the body. The eversion of this cylinder causes the ovipositor to be extruded some distance during oviposition. The gonopore opens posterior to the ninth sternite at the tip of the tubular ovipositor. Its opening is

bordered by a single palp, that of the right side being absent. Ventral to the cylinder formed by the invagination of the body-wall, and extending through the sixth and seventh segments is



TEXT-FIG. 31.

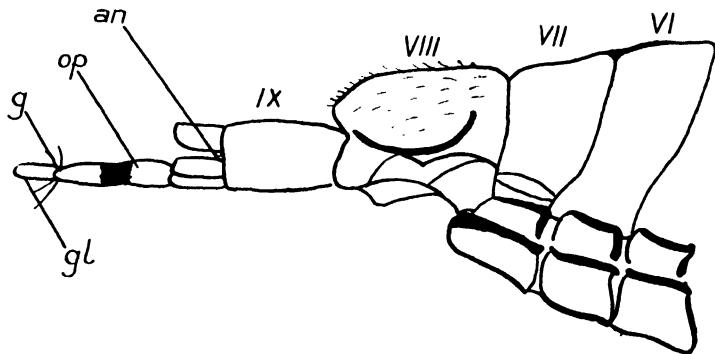
Adult structure of *Sitodrepa panicea* L. $\times 25$. *ag*, accessory gland; *c*, chitinous cylinder; *chr*, chitinous rod; *cov*, connective tissue of ovary; *dsp*, dorsal sac (spermatheca); *g*, gonopore; *gl*, genital palp; *od*, oviduct; *op*, ovipositor; *r*, rectum; *sp*, spermatheca; *ut*, uterus.

a Y-shaped chitinous rod, the fork of the Y being directed posteriorly. Powerful muscles serving to extend the posterior segments of the body during oviposition are attached to the rod.

(b) The Immature Insect.

(1) The Ovipositor. The Larva. In the larva nine distinct tergites and sternites are present. The anus is situated on a sclerite posterior to the ninth sternite, with a linear depression marking the position of the future genital aperture, immediately anterior to it. No genital appendages are present.

The Pupa. When pupation takes place, the body undergoes a certain amount of shortening, especially in the terminal abdominal segments. Nine tergites and sternites can still be



TEXT-FIG. 32.

Abdomen of *Sitodrepa panicea* L. with ovipositor extended.

× 40. *an*, anus; *g*, gonopore; *gl*, genital palp; *op*, ovipositor; *VI-IX*, sixth to ninth abdominal segments.

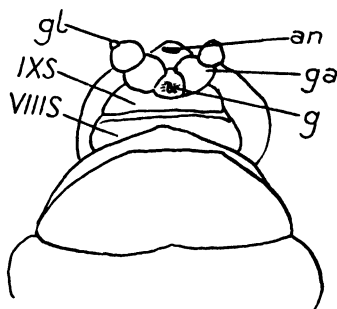
recognized, and with the straightening of the abdomen the anus moves caudad. The genital depression is now seen to lie between the bases of a pair of appendages of the ninth sternite which takes the form of a pair of two-jointed plates. The plate on the left side is provided with an apical palp; on the right side the latter is absent (Text-fig. 33). This asymmetry is a constant condition in the female.

Transverse sections through the posterior border of the ninth segment show that the appendages are outgrowths of the ectodermal layer bordering the genital depression. The latter takes the form of an invagination of the body-wall, at first shallow, but deepening as the pupa matures, its external opening forming the gonopore (fig. 34, Pl. 9). The genital appendages subsequently become fused together so that the

gonopore, at first situated between their bases, now moves posteriorly and comes to lie at the apex of the median appendage formed by their fusion (fig. 35, Pl. 9).

(c) The Efferent System.

The Larva. In the larva the only parts of the efferent system which can be recognized are the ovaries. These occupy a dorso-lateral position, one on each side of the alimentary tract



TEXT-FIG. 33.

Terminal abdominal segments of pupa of *Sitodrepa panicea* L. Ventral view. $\times 90$. *an*, anus; *g*, gonopore; *ga*, genital appendage; *gl*, genital palp; *VIII S*, eighth sternite; *IX S*, ninth sternite.

in the fourth and fifth abdominal segments. Each ovary is composed of six ovarioles which consist of a central mass of epithelial cells bounded by a basement membrane and an external coat of connective tissue. The epithelial cells are closely packed with well-marked nuclei.

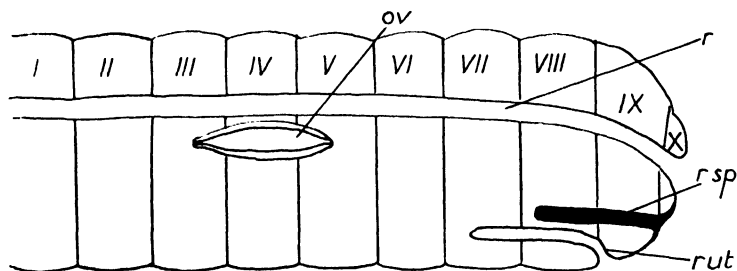
During the pre-pupal instar the ovaries elongate to some extent and now reach from the middle of the third to the middle of the fifth segments (Text-fig. 34). At this stage it is impossible to detect any trace of the oviduct.

The genital depression, as previously noted, is an invagination of the ectoderm, situated posterior to the ninth sternite. At first shallow, this invagination later sinks more deeply into the body-cavity and extends anteriorly to the middle of the eighth segment as a blind tube. This is the rudiment of the spermatheca, and its external opening, the future gonopore, is bordered

by the genital appendages (figs. 32, 33, and 34, Pl. 9; Text-fig. 34).

The uterus originates as an unpaired invagination of the ectodermal layer between the eighth and ninth sternites and extends below the spermathecal rudiment to the anterior border of the seventh sternite, where it ends blindly (Text-fig. 34).

In the pre-pupa, therefore, the terminal unpaired region of the efferent system is represented by two separate and distinct



TEXT-FIG. 34.

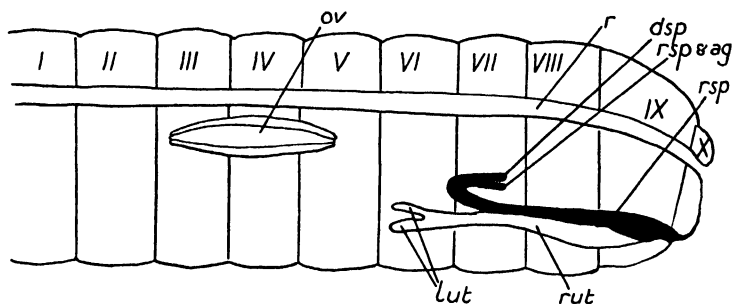
Schematic representation of longitudinal section through abdomen of pre-pupa of *Sitodrepa panicea* L. *ov*, ovary; *r*, rectum; *rsp*, rudiment of spermatheca; *rut*, rudiment of uterus; I-X, abdominal segments.

invaginations of the ectoderm, originating independently and having separate external openings.

The Pupa. In a young pupa the ducts from the ovarioles are seen to develop from the epithelial layer continuous with the distal posterior region of each ovariole. As development proceeds, the short ducts on either side unite to form the paired oviducts (figs. 36 and 37, Pl. 9). By the time the pupa is mature, communication between the blind ends of the paired oviducts and the paired uteri (*vide infra*) has been established.

Several changes may also be noted in the terminal region of the genital ducts. The spermathecal rudiment has widened and now extends to the anterior border of the seventh sternite, where it bends over, its free distal extremity being directed posteriorly and being divided longitudinally into two, the rudiments of the dorsal sac in the adult and of the functional

spermatheca and the accessory gland. The uterus has lost its external opening, the latter being completely closed over, its cavity has widened, and it now extends to the anterior border of the sixth sternite, where it bifurcates, the paired uteri thus formed ending blindly (fig. 31, Pl. 9). During the early stages of pupation, the rudiments of spermatheca and uterus approach one another near the anterior border of the eighth sternite, the ventral wall of the spermatheca being closely applied to the dorsal wall of the uterus. A little later, the intervening walls



TEXT-FIG. 35.

Schematic representation of longitudinal section through abdomen of young pupa of *Sitodrepa panicea* L. *dsp*, dorsal sac (spermatheca); *lut*, lateral or paired uterus; *ov*, ovary; *r*, rectum; *rsp*, rudiment of spermatheca; *rsp ag*, rudiment of spermatheca proper and accessory gland; *rut*, rudiment of uterus; I-X, abdominal segments.

break down, and communication between the cavities of the two ducts is freely established throughout their whole length, except for a very short region posterior to where the dorsal sac, accessory gland, and spermatheca end freely in the seventh segment (fig. 39, Pl. 9; Text-fig. 35).

The single sexual duct thus formed has, therefore, three regions:

- (1) An anterior region derived wholly from the rudiment of the uterus.
- (2) A posterior region derived wholly from the rudiment of the spermatheca.
- (3) A median region whose dorsal wall is derived from the

dorsal wall of the spermathecal rudiment, whose ventral wall is derived from the ventral wall of the uterine rudiment.

The gonopore is not therefore the primary aperture of the uterus, this having been closed over during development, but the opening of the spermatheca.

As the pupa matures, growth in the posterior unpaired region of the uterus results in the spermatheca and gland being carried anteriorly. The point of divergence of the paired uteri is also carried forward so as to cause the formation of a loop in these latter which extend into the fifth segment.

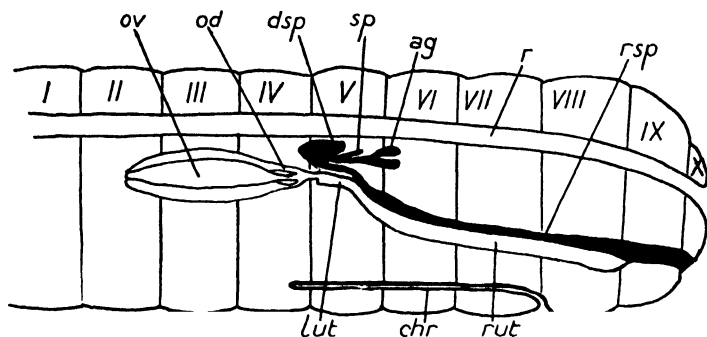
Communication between the blind ends of the paired uteri and the end of the mesodermal oviducts is not established until a later stage in the adult. The so-called oviducts of the adult are thus partly derived (in their anterior region) from the ducts developed from the posterior region of the ovaries, partly (in their posterior region) from the anterior branches of the uterus.

The chitinous rod arises late in the pupal instar as an invagination of the intersegmental membrane between the seventh and eighth sternites. It extends below the uterus as far as the sixth sternite. The ectodermal cells which line the invagination secrete a chitinous deposit which eventually forms the rod in the adult (fig. 42, Pl. 9; Text-fig. 36). The cavity of the invagination is at first open, but by progressive chitinization the mouth becomes closed over.

Histology. In the young pupa the spermathecal rudiment is of small diameter and is lined by a layer of large epithelial cells bounded by a basement membrane. No chitinous intima has yet been secreted (figs. 32 and 33, Pl. 9). The uterus is of a similar structure, but is smaller in diameter (figs. 31 and 32, Pl. 9).

In the mature pupa the three regions of the uterus are clearly defined. The anterior region and the paired uteri are of a large diameter, the epithelial cells are large and have well-marked nuclei. A well-developed muscular layer and a heavy deposit of chitin which almost obliterates the lumen of the duct are also present (fig. 38, Pl. 9). The median region has a characteristically pear-shaped outline and the dorsal and ventral regions are clearly defined. It closely resembles the anterior

region, but the cells of the epithelial layer are smaller, the chitinous deposit is less heavy, and the muscular layer is very well developed (fig. 39, Pl. 9). The dorsal sac is lined by a layer of small flattened epithelial cells. No muscular layer is developed and the cavity is filled with the chitinous secretion. The spermatheca proper is of a similar structure (fig. 41, Pl. 9). The accessory gland is lined by a layer of large irregular cells, the very dense contents of which obscure their outline. No muscular



TEXT-FIG. 36.

Schematic representation of longitudinal section through abdomen of mature pupa of *Sitodrepa panicea* L. *ag*, accessory gland; *chr*, chitinous rod; *dsp*, dorsal sac (spermatheca); *lut*, lateral or paired uterus; *od*, oviduct; *ov*, ovary; *r*, rectum; *rsp*, rudiment of spermatheca; *rut*, rudiment of uterus; *sp*, spermatheca proper; *I-X*, abdominal segments.

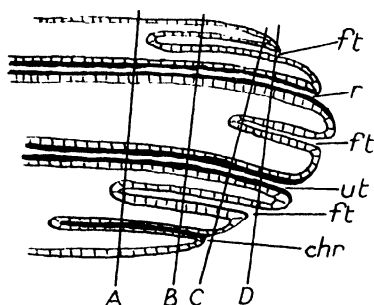
layer is present and the lumen is small (fig. 40, Pl. 9). The common duct of the spermatheca and accessory gland is provided with a muscular coat.

The posterior region of the uterus is much reduced in diameter. Its muscular layer is poorly developed (fig. 43, Pl. 9).

Maturation. Concurrent with the processes of development described above, the body segments are subjected to a process of chitinization. This however does not take place uniformly all over the body; the abdominal segments posterior to the eighth remain for a while membranous and flexible. While in this state the posterior region is subjected to a gradual telescoping process with the result indicated in Text-fig. 37.

The anus and gonopore thus come to lie very close together,

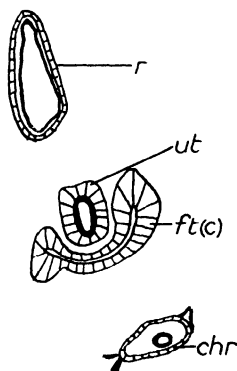
while the point of origin of the chitinous rod is again brought closer to the gonopore (Text-fig. 37). Owing to the fact that the gonopore is situated anterior to the anus, the body-wall appears to be more deeply sunken ventrally and reaches anteriorly further than dorsally. Transverse sections through a newly emerged adult in which the telescoping process is complete show this very clearly. The telescoped body-wall first makes its appearance at the posterior border of the seventh



TEXT-FIG. 37.

Diagrammatic longitudinal section through posterior region of adult of *Sitodrepa panicea* L. *chr*, chitinous rod; *ft(c)*, infoldings of body-wall due to telescopic process (chitinous cylinder); *r*, rectum; *ut*, uterus; Arrows A, B, C, D, represent transverse sections through the body shown in Text-figs. 38, 39, 40, and 41 respectively.

Diagrammatic transverse sections through posterior body segments in adult of *S. panicea* L. *chr*, chitinous rod; *ft(c)*, infoldings of body-wall (chitinous cylinder); *r*, rectum; *ut*, uterus.



TEXT-FIG. 38.

segment as a pair of blind tubes a little to each side of, and ventral to, the uterus. Each tube extends towards the middle line, where the two meet, their intervening walls breaking down to form a single crescent-shaped tube with a common, very narrow cavity (Text-fig. 38). More posteriorly, the horns of the crescent arch upwards, and finally unite dorsally in the eighth segment, above the rectum which now lies close to the uterus (fig. 43, Pl. 9; Text-fig. 39). Between the seventh and eighth

segments, the rectum and uterus are comparatively free, being only partially enclosed by the crescent; from the eighth segment to the tip of the abdomen, they are wholly enclosed within the double-walled tube formed by its closure. Posteriorly, a secondary in-sinking of the body-wall between the anus and the gonopore separates the rectum from the uterus and in transverse sections each appears to be enclosed within an epithelial tube of its own (Text-figs. 40 and 41). Distally the gonopore opens between the genital appendages posterior to the ninth sternite, with the anus posterior to the gonopore.

Histologically the tube is obviously of ectodermal origin and presents the following structure passing from within outwards (fig. 43, Pl. 9).

1. An epithelial layer of large irregular cells with cell-marked nuclei.
2. A cavity.
3. An epithelial layer, the cells of which are regular and not so large as in (1).
4. A poorly developed layer of circular muscles.

Secretion of chitin by the two epithelial layers takes place and a thin layer bounds the cavity (2) externally and internally.

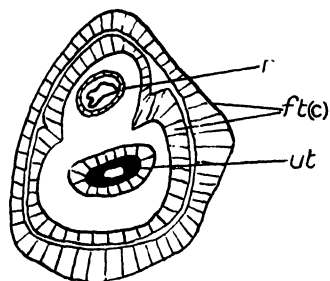
By various stages of increased chitinization the adult form is now reached. The inner wall of the telescoped posterior region becomes hard and rigid with a heavy deposit of chitin, the outer wall remaining membranous to a large extent.

(2) *Gastroidea polygona* L.

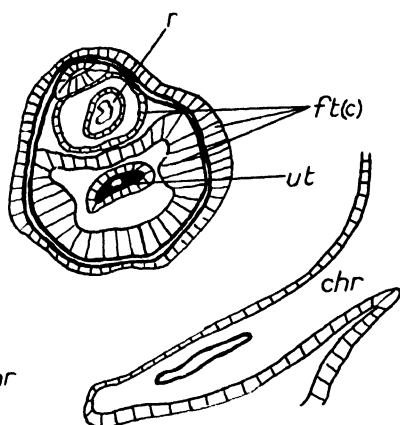
(a) Adult Structure.

In the mature female the abdomen is so very greatly distended by the large number of eggs that the sternites and tergites appear as isolated sclerites with much enlarged intersegmental membranes. The gonopore is a large, transverse slit posterior to the ninth sternite, and its aperture is bordered by a pair of widely separated appendages (Text-fig. 42).

The ovaries extend from the first to the third abdominal segments. The oviducts are short and very wide. They unite in the fifth segment to form the uterus which receives dorsally the common duct of the spermatheca and the accessory gland.

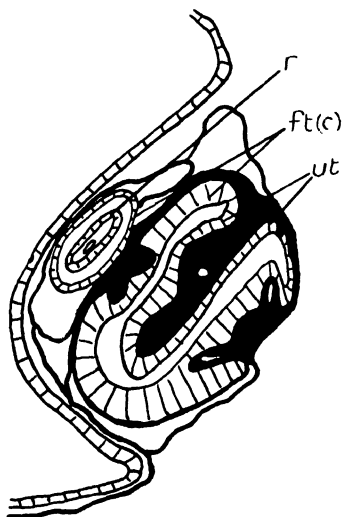


TEXT-FIG. 39.



TEXT-FIG. 40.

For description see Text-fig. 38.

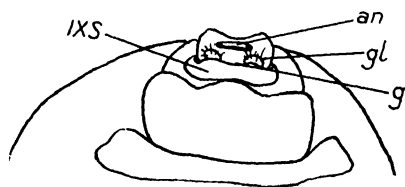


TEXT-FIG. 41.

For description see Text-fig. 38.

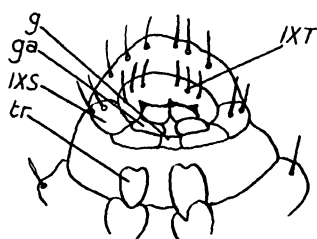
As a result of the distension of the abdomen, the telescoping of the posterior segments does not take place to any great extent; neither is the chitinous rod formed ventral to the uterus.

Owing to the large number of eggs present in the abdomen, the wide and thin-walled nature of the uterus, and the absence of highly chitinized parts, it is an extremely difficult matter to make a satisfactory dissection of the adult. The above description has, therefore, been compiled with the aid of serial sections taken through a mature pupa in which the eyes and mouth parts had acquired the colour and form of the adult.



TEXT-FIG. 42.

Terminal abdominal segments of adult of *Gastroides polygona* L., ventral view. $\times 28$. *an*, anus; *g*, gonopore; *gl*, genital palp; *IX S*, ninth sternite.



TEXT-FIG. 43.

Terminal abdominal segments of pupa of *G. polygona* L., ventral view. $\times 35$. *g*, gonopore; *ga*, genital appendage; *tr*, tarsus of third pair of legs; *IX T*, ninth tergite; *IX S*, ninth sternite.

(b) The Immature Insect.

(1) The Ovipositor. The Larva. In the female larva, as in the male, nine tergites and sternites and the reduced tenth segment can be recognized. Externally, there is no sign of genital appendages and the female larva is hence indistinguishable from the male.

The Pupa. After pupation has taken place, the female is easily recognized by its larger size, attaining to a length of 5 mm., while the male only measures 4-4½ mm.

The sexes may also be distinguished by the presence of genital appendages in the female.

Nine tergites and sternites can still be recognized and posterior to the ninth sternite is the wide-mouthed invagination of the genital duct. This is bordered by a pair of jointed appendages bearing palpi at their apices (Text-fig. 43). The

plates arise as diverticula of the ectodermal layer at the posterior border of the ninth sternite, and in the young pupa are massive and thick-walled (fig. 47, Pl. 9). In older pupae they become much reduced in size and remain distinct from each other with the palpi widely separated (fig. 49, Pl. 10).

(c) The Efferent System.

In the young pupa the ovaries occupy a dorso-lateral position extending from the third to the fifth abdominal segments. Each ovary is composed of numerous ovarioles. The oviducts are as yet undeveloped, but the terminal region of each ovariole assumes a duct-like structure, the cells of the epithelial layer being arranged around a central cavity.

The unpaired region of the genital duct arises quite early in the pupal instar as two separate invaginations of the ectoderm. The first invagination is formed immediately posterior to the eighth sternite and is the rudiment of the uterus. It extends as far as the anterior border of the seventh segment, where it divides to form two wide lateral ducts, the paired uteri. The uterine pore is very wide (figs. 44, 45, and 46, Pl. 9).

Posterior to the ninth sternite and at a slightly later stage of development a second invagination is formed. This extends only to the posterior border of the eighth segment and is the rudiment of the spermatheca. It is entirely separate from the uterine rudiment throughout its whole length (figs. 45, 46, and 47, Pl. 9).

As the pupa matures the ovaries undergo a forward migration, and eventually occupy a position extending from the middle of the first to the middle of the third abdominal segments. The ducts from the ovarioles unite to form a pair of oviducts, one on each side of the body. These extend at first from the middle of the second to the middle of the fourth segment. Here they diminish greatly in size, losing their lumina and having the appearance of solid cords of cells (fig. 51, Pl. 10).

Meanwhile, the rudiment of the uterus has elongated considerably and now extends to the middle of the sixth segment. Here it bifurcates and the paired uteri so formed meet the oviducts in the fourth segment. Although the blind ends of the

uteri and oviducts are apposed to one another (fig. 52, Pl. 10), communication between their cavities is deferred for a considerable period and does not take place until the end of the pupal instar.

The rudiment of the spermatheca now extends to the middle of the seventh segment, it becomes divided into two by a longitudinal furrow, the dorsal half going to form the accessory gland and the ventral half the tubular spermatheca. The rudiment of the gland soon becomes differentiated into a bag-like gland and a long and narrow duct.

In the eighth segment the spermathecal and uterine rudiments lie close together, and towards the posterior border of the segment their intervening walls break down and their cavities merge into one (fig. 48, Pl. 9). The uterus now loses all trace of its former opening posterior to the eighth sternite, the uterine pore being completely closed over during growth. The opening of the spermatheca posterior to the ninth sternite becomes the functional gonopore of the adult (fig. 49, Pl. 10).

Thus, as in *Sitodrepa panicea*, the unpaired portion of the sexual duct in the female has three primary divisions:

- (1) The anterior region derived wholly from the rudiment of the uterus;
- (2) The posterior region derived wholly from the rudiment of the spermatheca;
- (3) The median region, of which the dorsal wall is derived from the spermathecal rudiment, the ventral wall from the uterine rudiment.

At maturity the uterus and spermatheca have grown forwards to such an extent that the posterior unpaired region now extends as far as the anterior border of the seventh segment. The spermatheca and gland lie in the fifth segment. The paired uteri diverge at the anterior border of the fifth segment and their union with the paired oviducts takes place at the posterior border of the third segment. The oviduct is correspondingly shortened in length and the adjacent walls of uterus and oviduct have broken down, communication thus being established between the cavities of the two ducts.

Histology. Each ovariole is composed of a mass of epithelial

cells bounded by a delicate basement membrane. The oviducts are mesodermal in origin and are formed by the union of the short ducts from the ovarioles. They are lined by a layer of epithelium invested by a basement membrane (figs. 55, 56, and 57, Pl. 10).

In the young pupa the ectodermal layer of the spermatheca and uterus is composed of large regular cells, and the cavities of the invaginations are small (figs. 44-7, Pl. 9).

As the pupa matures the cavities of uterus and spermatheca widen considerably, the cells forming their walls secrete a chitinous lining and shrink noticeably (fig. 48, Pl. 9; fig. 52, Pl. 10).

The accessory gland has a wall composed of larger regular epithelial cells which have very dense contents. The cavity of the gland is small and is lined with the secretion of the epithelial layer (fig. 53, Pl. 10). Its duct is of a smaller diameter and its cells are small and regular. The wall of the tubular spermatheca is very similar to the duct of the gland (fig. 54, Pl. 10).

The absence of a well-developed muscular system in connexion with the uterus is noticeable, the latter remaining a very wide, thin-walled duct. The genital appendages do not become fused together. No telescoping of the posterior segments of the abdomen takes place here, and the elaborate double-walled cylinder, so conspicuous in sections and dissections of the females of *Sitodrepa panicea*, is entirely absent.

(3) *Anthonomus pomorum* L.

(a) Adult Structure (after Miles, 32).

'The female reproductive organs consist of two ovaries, one on each side of the body. Each ovary is composed of two ovarian tubes opening into a common oviduct which leads into the uterus.

'At the anterior end of each egg-tube a terminal filament is located; this serves to suspend, or connect, the ovaries with the strands of the fat-body.

'The oviducts at their posterior extremities lead into a common chamber, the uterus, and its lower portion, the vagina. The

bursa copulatrix runs into the uterus; it is connected with the receptaculum seminis and the accessory gland.

'At the posterior extremity of the vagina are two heart-shaped chitinous plates, produced posteriorly into two small rounded papillae. A chitinous rod runs from the median region of the uterus to the posterior extremity of the vagina. The function of the chitinous plates is suggested to be the guidance of the ovum at oviposition.'

In the above description by Miles the 'bursa copulatrix' corresponds to the 'dorsal sac' opening into the uterus in *Sitodrepa panicea* and *Gastroidea polygona*. Whether this structure actually functions as a bursa copulatrix is as yet undecided, and, therefore, the use of this term has not been adopted in the present study. The 'receptaculum seminis' corresponds similarly to the 'spermatheca'. The 'heart-shaped chitinous plates' with their 'apical papillae' represent the genital appendages which in this species do not become fused together.

(b) The Immature Insect.

(i) The Ovipositor. Nine abdominal tergites and sternites are present in the larva. A sclerite representing the tenth segment intervenes between the tergite and sternite of the ninth segment and bears the anus. No genital appendages are present.

After pupation has taken place nine tergites and sternites are still recognizable. As in the male, the ninth tergite is prolonged into a pair of caudal spines.

Posterior to the ninth sternite is a depression bordered by a pair of appendages. The depression marks the opening of the spermathecal invagination. The appendages are small plates bearing palpi at their apices (Text-fig. 44).

In transverse sections the genital appendages are seen to be diverticula of the ectodermal layer of the ninth sternite.

(ii) The Efferent System. Until the last stages of the pupal stadium the ovaries and oviducts are very little developed. Miles observes that in young adults soon after emergence the egg-tubes are quite undeveloped, and remain so until the following spring.

The rudiment of the ovary consists of a pair of ovarioles which at this stage of development take the form of delicate cords of epithelial cells. Each ovariole is invested by a sheath of connective tissue and extends from the middle of the first to the middle of the second abdominal segment. In its posterior region the epithelial cells of the ovariole become arranged around a small cavity to form a very slender duct (fig. 60, Pl. 10).

The rudiment of the uterus may first be observed during the pre-pupal instar. Enlarged ectodermal cells form a thickened plate extending for half the length of the eighth sternite. A groove is formed, commencing posteriorly, and gradually extending the length of the plate (fig. 55, Pl. 10). Eventually the groove becomes converted into a tube opening posterior to the eighth segment.

A similar plate of cells in the ninth segment gives rise to the rudiment of the spermatheca, its aperture being bordered by the genital appendages (fig. 56, Pl. 10). The mode of development of the uterus and spermatheca is thus essentially similar to that noted in the previously described species.

During the pupal stadium the uterine rudiment grows forward into the fourth abdominal segment, where it divides to form the paired uteri. The union of the paired uteri and oviducts was not observed in any of the sections obtained, and is evidently deferred for a longer period than in other species.

The spermathecal rudiment also grows forward, and its blind anterior end becomes divided into two tubes which are destined to form the functional spermatheca and accessory gland of the adult.

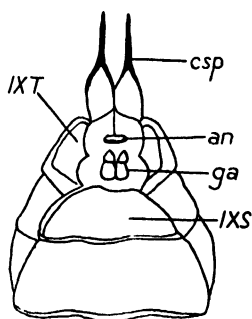
In the course of development the rudiments of uterus and spermatheca approach one another, and finally their intervening walls break down, so that a common cavity and one composite duct are formed. The original opening of the uterus becomes closed over and lost, and in the adult the contents of the uterus are discharged into the spermatheca, the original opening of which functions as the gonopore (fig. 57, Pl. 10). T

The chitinous rod which acts as a support to the uterus and to which powerful muscles are attached, originates as a ventral invagination of the ectodermis between the seventh and eighth

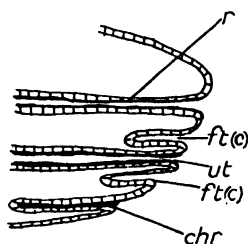
sternites. This invagination extends anteriorly into the fifth segment, and the rod is formed within it by the secretion of chitin (fig. 57, Pl. 10).

Histology. The ducts from the ovarioles are mesodermal structures, being lined by epithelium and invested by a sheath of connective tissue (fig. 60, Pl. 10).

The paired uteri and other structures of ectodermal origin are composed of a layer of regular cells and their cavities are lined by a chitinous secretion. In the paired uteri this secretion



TEXT-FIG. 44.



TEXT-FIG. 45.

Terminal abdominal segments of pupa of *Anthonomus pomorum* L., ventral view. $\times 50$. *an*, anus; *csp*, caudal spine; *ga*, genital appendage; *IX T*, ninth tergite; *IX S*, ninth sternite.

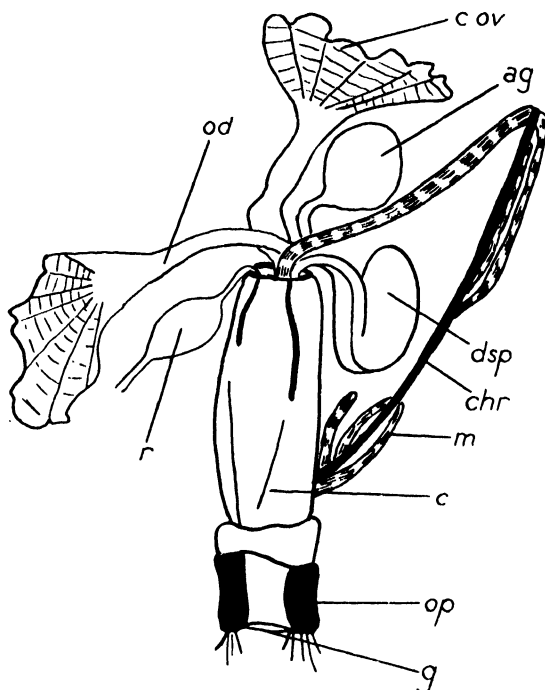
Diagrammatic longitudinal section through posterior segments of adult of *A. pomorum* L. *chr*, chitinous rod; *ft(c)*, infoldings of body-wall (chitinous cylinder); *r*, rectum; *ut*, uterus.

almost fills the cavity of the duct; in the other ducts it is present as a thin layer. The median uterus and spermatheca are provided with outer coats of circular muscles which are not present in the paired uteri and the accessory gland.

Towards the posterior region of the abdomen and the body-wall becomes sunken in around the uterus as shown in Text-fig. 45. This telescoping does not affect the rectum, and in transverse sections the uterus appears to be surrounded by a double-walled tube of ectodermal origin (fig. 58, Pl. 10).

Anthonomus pomorum, therefore, appears to be a type intermediate between *Sitodrepa panicea* and *Gas-*

troides polygoni; the telescoping of the posterior abdominal segments having taken place to a less extent than in the former, to a greater extent than in the latter.



TEXT-FIG. 46.

Adult structure of *Rhagium bifasciatum* F. $\times 8$. *ag*, accessory gland; *c*, chitinous cylinder; *chr*, chitinous rod; *cov*, connective tissue of ovary; *dsp*, dorsal sac (spermatheca); *g*, gonopore; *m*, muscle; *od*, oviduct; *op*, ovipositor; *r*, rectum.

(4) *Rhagium bifasciatum* F.

(a) Adult Structure (Text-figs. 46 and 47).

Two large ovaries are present, each being provided with a short and wide oviduct. The oviducts open into the paired uteri which by their union form the median uterus. In the posterior region of the abdomen the latter enters, in company with the rectum, a strongly chitinized double-walled cylinder

formed by the telescoping of the abdominal segments posterior to the seventh. A dorsal sac which receives the duct of an unpaired gland opens into the uterus.

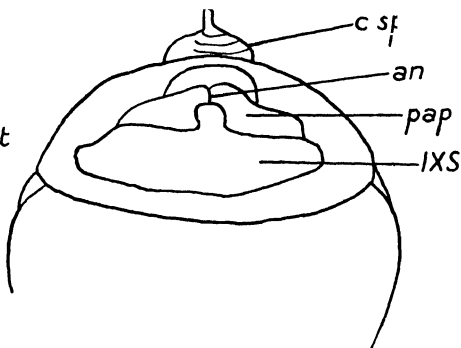
The ovipositor is a short and stout chitinized tube, its origin from a pair of appendages being indicated by a longitudinal dorso-ventral suture. The gonopore is situated between a pair of small, club-shaped palpi.

Ventral to the uterus is a strongly chitinized Y-shaped rod



TEXT-FIG. 47.

Ovipositor of *Rhagium bifasciatum* F. $\times 22$. *ct*, spines; *g*, gonopore; *gl*, genital palp; *ls*, longitudinal suture; *op*, ovipositor.



TEXT-FIG. 48.

Terminal abdominal segments of larva of *R. bifasciatum* F., ventral view. $\times 15$. *an*, anus; *csp*, caudal spine; *pap*, anal papilla; *IXS*, ninth sternite.

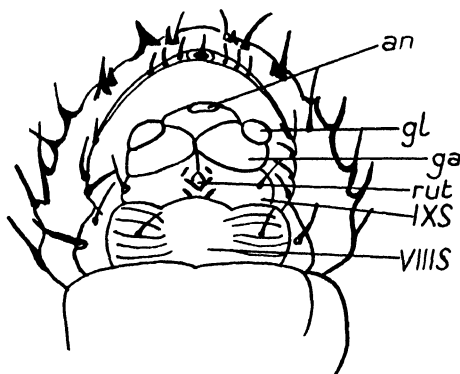
attached to the body-wall and to the chitinized cylinder by powerful muscles. The whole structure closely resembles that of the reproductive system in *Sitodrepa panicea*.

(b) The Immature Insect.

(i) The Ovipositor. In the larva nine tergites and sternites are present. Posterior to the ninth segment is a sclerite which is produced dorsally into a short spine. This sclerite bears the anus which is bordered by a pair of fleshy papillae. No genital appendages are visible externally, even in the largest of larvae examined; neither can any invagination indicating the position of either the spermatheca or the uterus be detected (Text-fig. 48).

In the pupa the same number of tergites and sternites can still be recognized. The ninth segment bears posteriorly a pair of large appendages, each of which is provided with an apical palp. Immediately anterior to the bases of the appendages the ninth sternite is marked by a longitudinal depression which forms the opening of the uterine invagination (Text-fig. 49).

On the emergence of the imago the number of visible abdominal segments is seen to have been reduced to seven. The ovipositor protrudes between the seventh sternite and tergite



TEXT-FIG. 49.

Terminal abdominal segments of pupa of *Rhagium bifasciatum* F., ventral view. $\times 35$. *an*, anus; *ga*, genital appendage; *gl*, genital palp; *rut*, uterine invagination; *VIII S*, eighth sternite; *IX S*, ninth sternite.

and is seen to have been formed by the fusion of the appendages of the ninth segment, their apical palps remaining free. The fate of the segments posterior to the seventh is evident as soon as a dissection of the female has been made. These segments have been retracted into the body to form the double-walled cylinder which encloses the rectum and uterus. This cylinder does not form the ovipositor, the latter being of a true appendicular nature, but merely serves as a mechanism whereby it may be withdrawn into the body or extruded therefrom (fig. 62, Pl. 10).

(ii) The Internal Ducts. In the larva there is little development of the ovaries or the oviducts.

In the pupa, while lack of material rendered it impossible to trace the development step by step, several interesting points could be noted in sections.

The ovaries and the oviducts leading therefrom are mesodermal in origin. Each ovary is composed of several ovarioles lined by a layer of epithelium and invested by a sheath of connective tissue (fig. 63, Pl. 10). In the young pupa the oviducts are quite solid (fig. 64, Pl. 10).

As in the females of the other species examined, the functional uterus of the adult is derived from two primary invaginations of ectodermal origin which subsequently fuse to form a single duct. The uterine pore is easily observed in young pupae, being situated posterior to the eighth segment. In older pupae this primary aperture is closed over and the opening of the spermatheca may be observed between the bases of the genital appendages immediately posterior to the ninth segment. Later, by the fusion of the latter, the gonopore moves posteriorly to become situated between the palpi at their apices.

Sections through a mature pupa show clearly that the posterior region of the uterus arises as a single tube of ectodermal origin. It has a conspicuous lining of chitin and is invested by a well-developed coat of circular muscles. This region of the uterus is derived wholly from the original spermathecal invagination arising posterior to the ninth segment. In this posterior region also, the uterus is, with the rectum, enclosed in a double-walled cylinder of ectodermal origin. The manner of appearance of this cylinder, and its structure, are very similar to those of the cylinder present in *Sitodrepa panicea*. It is evidently formed by the telescoping of the body segments posterior to the seventh (fig. 62, Pl. 10).

Anterior to its enclosure in the chitinous cylinder the uterus becomes clearly divisible into two regions, both of ectodermal origin and of similar structure (fig. 61, Pl. 10). The dorsal region represents the spermatheca of the adult, and is derived from the anterior extremity of the original spermathecal rudiment. Anteriorly, it becomes divided, the one division forming the duct of the accessory gland, the other the functional spermatheca.

The ventral region represents the uterus of the adult, and is

derived from the primary uterine invagination which is formed immediately posterior to segment eight. Some little distance anterior to its separation from the spermatheca, the uterus divides into two lateral branches. These extend to the posterior region of the oviducts, becoming applied to the blind ends of the latter. Communication between the cavities of the oviduct and uterus is not established until a later stage in development (fig. 64, Pl. 10).

The chitinous rod is formed, as in other species, as an invagination posterior to the seventh sternite (fig. 61, Pl. 10).

D. CONCLUSIONS.

From the foregoing account of the structure and development of the female reproductive system in *Sitodrepa panicea*, *Gastroidea polygona*, *Anthonomus pomorum*, and *Rhagium bifasciatum*, the following conclusions may be drawn:

(1) The Ovipositor and Associated Structures.

The ovipositor in the Coleoptera is derived from a pair of palp-bearing plates, which may or may not fuse to form a median tubular organ bearing the gonopore. From their position, mode of origin and structure, these plates appear to be homologous with the lateral ovipositor lobes in other Insecta, and hence represent the coxites of the ninth segment with their styli.

There appears, however, to be some foundation for Verhoeff's statement in 1893 (50), that the posterior abdominal segments are modified in relation to oviposition. The ovipositor itself has been shown to be of an appendicular nature, but in some species of Coleoptera, e.g. *Sitodrepa panicea*, *Rhagium bifasciatum*, the intersegmental membranes between the posterior abdominal segments eight to ten undergo an increase in length. While still membranous and flexible, this region becomes telescoped into the body-cavity and is subjected to a process of chitinization, the outer wall remaining membranous, the inner wall forming a stiff chitinous cylinder. Powerful muscles are developed for the eversion and retraction of these segments. When the structure is retracted, the uterus

and rectum appear to be enclosed within a double-walled cylinder. By the eversion of this cylinder the ovipositor becomes thrust well outside the body at the tip of the long and tapering tube formed by the posterior segments. This is well illustrated in Text-fig. 32.

Moreover, there seems to be some correlation between the extent to which this telescoping takes place, the degree of fusion between the genital appendages, and the mode of oviposition. For example, in *Gastroidea polygoni*, where the eggs are deposited in rows on the surfaces of leaves, and the abdomen is very much distended with eggs at the time of oviposition, little or no withdrawal of the posterior segments takes place; the uterus and rectum are free to the tip of the abdomen, and the genital appendages have the appearance of reduced plates bordering the gonopore. Here there seems no need for the extrusion of the body to facilitate the deposition of the eggs, and, to quote Verhoeff: 'Where the eggs are scattered broadcast, no ovipositor (i.e. no telescoping of the body segments) is present.'

In *Sitodrepa panicea* and *Rhagium bifasciatum*, on the contrary, the double-walled cylinder enclosing rectum and uterus is most noticeable in sections through the abdomen of the pupa and in dissections of the mature adult. In the former species the eggs are deposited in cracks and grooves in the substratum, in the latter in the wood of coniferous trees. The prolongation of the abdomen into a narrow retractile tube, together with the fusion of the appendages to form a tubular ovipositor, is apparently advantageous.

The case of *Anthonomus pomorum*, however, presents a difficulty. According to Miles, the female weevil eats a hole in one anther lobe of a blossom, and then extends her ovipositor, thrusting it down into the prepared aperture. In the females of *Anthonomus* which were examined it was found that the prolongation of the body was very slight, and that when retraction had taken place, there was no close connexion between the body-wall and the uterus and rectum. Moreover, the genital appendages remain free and do not fuse to form a tubular ovipositor.

While no hard and fast rule can be laid down, therefore, that the act of oviposition is facilitated in the manner suggested, or that the modifications noted are correlated with function, yet in the four species studied, three at least of the types suggested by Verhoeff appear to be present, namely:

- (a) The 'Legerohre' for the deposition of the eggs in the bark or the wood of trees, e.g. *Rhagium bifasciatum*.
- (b) The 'Legesabel' for the deposition of eggs in the soft tissues of young shoots, e.g. *Anthonomus pomorum*.
- (c) The absence of any special modification when the eggs are to be scattered broadcast, e.g. *Gastroidea polygoni*. The case of *Sitodrepa panicea* seems to approximate most nearly to that of *Rhagium*, viz. to the 'Legerohre'.

Finally, the mode of oviposition in connexion with the structure of the ovipositor and the terminal abdominal segments must be studied in a great many species before a definite statement on the subject can be pronounced or the possible value in taxonomy estimated.

The chitinized rod ventral to the uterus originates as an invagination of the body-wall posterior to the seventh sternite. As this rod is already in existence when the full complement of body segments can still be recognized, it cannot represent the part or the whole of a converted body segment. Whether the homologue of this rod is present in other Insecta is an open question. A similar ectodermal invagination posterior to the seventh segment is described by George (20) in *Philaenus spumarius*. This goes to form the anterior region of the common oviduct (i.e. uterus), the posterior portion being derived from the ectoderm of the eighth segment. George also describes an invagination of the ninth segment which goes to form the accessory gland. It is possible that the line of evolution in the Coleoptera has been such that the anterior region of the common oviduct has been derived from the duct originating posterior to the eighth segment, its posterior region from the duct originating posterior to the ninth. The duct originating

posterior to the seventh segment thus loses all connexion with the efferent system and is converted into the chitinous rod.

(2) The Efferent System.

(a) There appears to be a shortening of the original efferent passage noted by Korschelt and Heider so that the oviducts are partly derived from the mesoderm, partly from the ectoderm. The extent of the ectodermal portion, which consists of a pair of outgrowths from the uterus, is indicated by the conspicuous lining of chitin which is present.

(b) All other parts of the efferent system, viz. uterus, spermatheca, and accessory gland, are ectodermal in origin. This point may be confirmed by direct observation of their development as well as by the study of their histological structure. Singh Pruthi, Nussbaum, Jackson, and others are in agreement as to this point, while the views of Herold and Balbiani are in opposition.

(c) All parts of the efferent system, with the exception of ovaries and oviducts, are unpaired in origin.

No paired rudiments of uterus, spermatheca, or gland were observed, the azygos condition being primary and not secondary as Nussbaum concluded.

In this matter Wheeler states that the vagina in the Orthoptera is derived from an unpaired invagination originating between the seventh and eighth sternites. Singh Pruthi, Nel, and George also find that the uterus is unpaired in origin.

Jackson appears to effect a compromise, for, while the uterus according to him is unpaired throughout its length, the accessory or sebaceous gland is paired in origin, while the spermatheca and receptaculum seminis are derived from a pair of vesicles situated posterior to the eighth segment. These vesicles fuse, losing all trace of their original paired nature, and subsequently give rise to the spermatheca and receptaculum seminis, one behind the other in the median line.

Verson and Bisson regard all parts of the efferent system as derived from the fusion and secondary modification of two pairs of ectodermal vesicles, one pair situated posterior to the eighth,

the other to the ninth, segment. Their work seems to have been influenced greatly by the conclusions of Jackson.

(d) The spermatheca and uterus arise independently, the one behind the ninth, the other behind the eighth segment. Communication between the two is established secondarily.

This is in accordance with the views of Jackson and Singh Pruthi, but in contradiction to those of Balbiani, who describes the spermatheca as arising from an outgrowth of the uterus.

In discussing Jackson's work, Singh Pruthi makes the mistake of referring to him as an author who regards the spermatheca as an outgrowth of the uterus itself. According to the original text Jackson describes the paired vesicles which subsequently give rise to the spermatheca and vesicula seminalis as being already in existence when the uterus first makes its appearance. It is true that the rudiments of the spermatheca and uterus are from the first in connexion with each other, as Singh Pruthi states, but Jackson describes very definitely the mode of development of both structures, and it is clear that he regards each as derived separately from the ectodermis.

(e) The rudiment of the uterus, which arises posterior to the eighth sternite, is homologous with the genital duct in those of the Insecta in which the gonopore is located posterior to the eighth sternite, and the primary opening of the uterus which subsequently becomes closed over and lost, is the homologue of that gonopore.

The functional gonopore of the Coleoptera, viz. the original aperture of the spermathecal rudiment, posterior to the ninth sternite, cannot be considered homologous with the gonopore in such Insecta where the latter is derived from the primary uterine pore.

There thus appears to be a progressive migration of the gonopore in the Insecta from posterior to the seventh, to the eighth, and finally to the ninth, segments. Singh Pruthi suggests that in the first case the gonopore represents the aperture of the true mesodermal oviduct. George, however, describes an ectodermal invagination arising posterior to the seventh sternite, the aperture of which becomes closed over in growth. Nel also suggests that the primary location of the aperture of the

unpaired oviduct (ectodermal) is posterior to the seventh sternite. There seems to be some confirmation for this suggestion in the study of the development of the chitinous rod in the Coleoptera.

Where the gonopore is located posterior to the eighth sternite it represents the aperture of the uterus, where posterior to the ninth, of the spermatheca. Christophers (Diptera) describes the origin of the uterus posterior to the eighth segment, with the invagination of the spermatheca in close connexion. The 'caecus' arises as an invagination posterior to the ninth segment. At a later stage of development these three structures all open into a common atrium. Now, if it be supposed that the invagination posterior to the ninth segment in the Diptera represents the spermathecal invagination, that in the course of development communication between the 'caecus', 'spermatheca', and uterus be established, the caecal opening being closed over and the uterine pore functioning as the gonopore; then the similarity between the mode of development and the homologies of the female reproductive system in the Diptera and the Coleoptera are at once clear.

With reference to the same point Jackson seems to have been in possession of the facts, but to have misinterpreted their significance. The 'posterior pair of vesicles' situated in the ninth segment and giving rise to the sebaceous glands is evidently the spermathecal rudiment, the 'anterior pair of vesicles' in the eighth segment corresponding to the uterine rudiment. The process which he describes as the 'backward migration of the gonopore' is thus seen to be the establishment of communication between the rudiments of uterus and spermatheca.

PART III. THE HOMOLOGIES OF THE MALE AND FEMALE REPRODUCTIVE SYSTEMS IN THE COLEOPTERA.

Parts I and II of the foregoing study would be incomplete without some attempt to analyse the homologies that exist between the male and female reproductive systems. Each sex has been considered separately and its homologies discussed, it

now remains to make a comparison between the two sexes. Various difficulties attend this task, since mode of origin, growth and development, as well as adult morphology, must be taken into consideration. The following conclusions, however, seem justifiable.

A. THE GENITAL APPENDAGES, VIZ. COPULATORY APPARATUS
AND OVIPOSITOR.

Since the intromittent organ (median and lateral lobes of the aedeagus) represents the endopodites of the ninth segment, while the ovipositor is derived from the coxites and styli of that segment, the male and female genital appendages in the Coleoptera cannot be regarded as homologous.

This conclusion has already been arrived at by Singh Pruthi (42-5).

In other orders of the Insecta where the three pairs of primary appendages are present in the female, and two pairs in the male, the appendages of the ninth segment, viz. the 'telopodites' and 'coxites' in both sexes are homologous. The homologues of the anterior pair of ovipositor lobes in the female, viz. the telopodites or endopodites of the eighth segment, are never present in the male.

While some authors such as Zander (58-61) will admit no relationship between the appendages of the two sexes, even of those borne by the ninth segment, others, such as Wheeler (57), Kershaw and Muir (27), make an attempt to show that in the male as well as in the female three pairs of primary appendages are present.

Zander states emphatically: 'dass die Stachelapparat und die männlichen Geschlechtsanhänge weder in toto noch in ihren Theilen irgend welche morphologische Übereinstimmung erkennen lassen. Beide sind total differente Bildungen.'

This seems unnecessarily sweeping, and Singh Pruthi considers that Zander's results indicate that the appendages in male Hymenoptera represent the endopodites and coxites of the ninth segments, and are hence homologous with the dorsal and lateral ovipositor lobes. It must be borne in mind, however, that Zander does not consider the genital appendages to be

serially homologous with segmental appendages, and hence sees no reason why any relation between the appendages of the two sexes should be considered to exist.

Wheeler, at the other extreme, homologizes the male and female appendages with the styli of the eighth, ninth, and tenth segments, the last pair having undergone a forward migration in the course of development. More recently, Kershaw and Muir describe three pairs of appendages in the Homoptera which 'arise in exactly the same place as in the female', viz. one pair on the eighth sternite and two pairs from an area which appears to be the enlarged eighth-ninth intersegmental membrane. They also consider the first pair of appendages to represent the coxites of the eighth segment---'while there is no evidence to show that g 3 (i.e. the homologues of the lateral ovipositor lobes) are the coxites of the ninth'.

Their zeal for an exact comparison between the male and female leads them to locate the male gonopore in the Homoptera between the eighth and ninth sternites, whereas all other investigators place it posterior to the ninth sternite. Apart from the fact that Singh Pruthi, working on the same group, found but two pairs of appendages on the ninth segment of the male, the above quotation shows that in their conclusions Kershaw and Muir differ essentially from other investigators; the anterior pair of ovipositor lobes is not considered homologous with the coxites of the eighth segment, but with the endopodites thereof (Verhoeff (49-53), Walker (55 and 56), Crampton (7-15), Singh Pruthi).

For these reasons the conclusions of Kershaw and Muir are not considered to shed any new light on the subject.

It is considered rather that with regard to the genitalia of the Insecta no general statement can be made, but that the condition prevailing in any particular group must be decided on after careful consideration. In this way only can the significance of the structures be realized and harmony be restored.

B. THE EFFERENT SYSTEM.

While the ovaries and testes are strictly homologous structures, the oviducts and vasa deferentia cannot be entirely

considered as such. The original efferent passages derived from the posterior region of the ovarian and testicular rudiments have, in the Coleoptera, undergone a greater or less amount of shrinkage, e.g. in the males of *Sitodrepa panicea*, *Gastroidea polygoni*, and *Anthonomus pomorum* the mesodermal vasa deferentia are reduced considerably in length and their terminal regions are supplemented by outgrowths from the ejaculatory duct. A similar process seems to have taken place in the females of all species examined, the terminal regions of the mesodermal oviducts being replaced by outgrowths from the uterus. Those portions of the vasa deferentia and oviducts which are derived wholly from the mesodermal rudiments are homologous structures; the ectodermal portions of the paired ducts must, however, be considered separately.

In the female the ectodermal regions of the oviducts are derived from outgrowths of the uterus; in the male the corresponding regions of the vasa deferentia from ectodermal structures, the origin of which is disputed.

According to Singh Pruthi, in his account of the development of *Tenebrio molitor*, the vasa deferentia, or paired ejaculatory ducts as he calls them, are wholly ectodermal in origin and are homologous with the paired ejaculatory ducts in the Homoptera. These latter develop from ectodermal ducts originating near, though not actually opening at, the posterior margin of the eighth sternite. From these facts Singh Pruthi concludes that the paired ejaculatory ducts are homologous with the uterine rudiment which arises in the female posterior to the eighth sternite. Since the uterus is unpaired in origin this seems to be a doubtful comparison.

If the gonopores in the Coleoptera are homologous, as seems likely, since both are located posterior to the ninth sternite, then the primary ducts, of which the gonopores are the apertures, namely, the ejaculatory duct and the spermathecal rudiment, are homologous structures. So much is admitted by Singh Pruthi. But, in *Sitodrepa panicea*, *Gastroidea polygoni*, and *Anthonomus pomorum*, it was found that the paired ejaculatory ducts arise as outgrowths from the

median ejaculatory duct as was suggested by Muir (34), and therefore can have no relation to the uterus which is derived from posterior to the eighth sternite and hence to the paired uteri which arise as outgrowths from the latter.

It appears, therefore, that in the Insecta, when the gonopore in the female is located posterior to the ninth segment, it may be considered to be the homologue of the male gonopore, each being derived from the aperture of a primary invagination located posterior to the ninth segment. Where the primary uterine pore functions as the gonopore of the adult, it cannot be regarded as strictly homologous with the opening of the ejaculatory duct.

In some cases, e.g. the Diptera, the homologue of the male gonopore is at first present as the aperture of the 'caecus', but this later becomes closed over, or moves forwards, the uterine pore functioning as the gonopore.

Some Lepidoptera retain both uterine and spermathecal apertures in the adult, while in the Coleoptera a third type of specialization is met with, the uterine pore being closed over and lost, while the spermathecal opening is the functioning gonopore of the adult.

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EXPLANATION OF PLATES 7-10.

LETTERING.

ag, accessory gland; *b*, bend in posterior region of ejaculatory duct; *bm*, basement membrane; *c*, chitinous cylinder; *ca*, outer wall of chitinous cylinder; *cb*, inner wall of chitinous cylinder; *ch*, chitinous intima; *chr*, chitinous rod; *conn*, connective tissue; *dsp*, dorsal sac (spermatheca); *ep*, epithelial layer; *g*, gonopore; *ga*, genital appendage; *gc*, clefts separating off genital appendage from genital pocket; *gl*, genital palp; *gp*, genital pocket; *hp*, ectodermal plate in eighth sternite; *l*, lumen; *lejd*, lateral ejaculatory duct; *ll*, lateral lobe; *lut*, lateral or paired uterus; *m*, muscle; *mejd*, median ejaculatory duct; *mejda*, anterior region of *mejd*; *mejdb*, median region of *mejd*; *mejdc*, posterior region of *mejd*; *ml*, median lobe; *nut*, median uterus; *nu*, nucleus; *od*, oviduct; *put*, uterine pore; *r*, rectum; *rsp*, spermathecal rudiment; *rut*, uterine rudiment; *s*, space between inner and outer walls of chitinous cylinder; *sec*, secretion of epithelial layer; *sp*, spermatheca; *sp.g*, rudiment of spiculum gastrale; *t*, transition between anterior and median regions of ejaculatory duct; *t'*, transition between median and posterior regions of ejaculatory duct; *te*, testis; *tf*, testicular follicle; *vd*, vas deferens; *ve*, vas efferens; *vs*, vesicula seminalis.

PLATE 7.

Figs. 1-9.—*Sitodrepa panicea* L. The Male.

Figs. 1-4.—Transverse Sections through the Pupa from Anterior to Posterior.

Fig. 1.—The testes and accessory glands. $\times 80$.

Fig. 2.—Origin of the lateral ejaculatory ducts and accessory glands. $\times 260$.

Fig. 3.—Anterior border of the genital pocket at the base of the genital appendages. $\times 200$.

Fig. 4.—The genital appendages. $\times 200$.

Figs. 5-9.—Histology of the Efferent System.

Fig. 5.—Testicular follicle. $\times 440$.

Fig. 6.—Vas efferens. $\times 440$.

Fig. 7.—Junction of vas efferens and vas deferens. $\times 440$.

Fig. 8.—Portion of wall of accessory gland. $\times 580$.

Fig. 9.—Median ejaculatory duct. $\times 580$.

Figs. 10-13.—*Gastroidea polygona* L. The Male.

Figs. 10-12.—Transverse Sections through Pre-pupa from Anterior to Posterior.

Fig. 10.—Origin of lateral ejaculatory ducts and accessory glands. $\times 130$.

Fig. 11.—Apex of genital pocket showing clefts separating off the genital appendages. $\times 130$.

Fig. 12.—The genital appendages. $\times 130$.

Fig. 13.—Young Pupa. Transverse Section showing Growth in the Anterior Region of the Ejaculatory Duct. $\times 130$.

PLATE 8.

Figs. 14–21.—*Gastroidea polygoni* L. The Male.

Figs. 14–16.—Transverse Sections through the Mature Pupa from Anterior to Posterior.

Fig. 14.—Median and lateral ejaculatory ducts, glands, vesicula seminalis, and vas deferens. Section indicated by arrow A in Text-fig. 22. $\times 130$.

Fig. 15.—Loop in posterior region of ejaculatory duct. Section indicated by arrow B in Text-fig. 22. $\times 130$.

Fig. 16.—Junction of median and posterior regions of ejaculatory duct. Section indicated by arrow C in Text-fig. 22. $\times 130$.

Figs. 17–21.—Histology of the Efferent System.

Fig. 17.—Testis and vas deferens. $\times 800$.

Fig. 18.—Vesicula seminalis. $\times 600$.

Fig. 19.—Accessory gland. $\times 900$.

Fig. 20.—Median region of ejaculatory duct. $\times 580$.

Fig. 21.—Posterior region of ejaculatory duct. $\times 1100$.

Figs. 22–30.—*Anthonomus pomorum* L. The Male.

Figs. 22 and 23.—Transverse Sections through the Pre-pupa from Anterior to Posterior.

Fig. 22.—Origin of lateral ejaculatory ducts and accessory glands. $\times 200$.

Fig. 23.—Gonopore and genital appendages. $\times 200$.

Figs. 24, 25, and 26.—Transverse Sections through the Pupa from Anterior to Posterior.

Fig. 24.—Testes, vas deferens, glands, ejaculatory duct, and vesicula seminalis. $\times 170$.

Fig. 25.—Origin of lateral ejaculatory ducts. $\times 190$.

Fig. 26.—Genital pocket and spicule. $\times 200$.

Figs. 27–30.—Histology of the Efferent System.

Fig. 27.—Testes and vas efferens. $\times 440$.

Fig. 28.—Vas deferens. $\times 440$.

Fig. 29.—Accessory gland. $\times 770$.

Fig. 30.—Median ejaculatory duct. $\times 730$.

PLATE 9.

Figs. 31–43.—*Sitodrepa panicea* L. The Female.

Figs. 31–4.—Transverse Sections through a Young Pupa. Anterior to Posterior.

Fig. 31.—The anterior forking of the uterine rudiment. $\times 200$.

Fig. 32.—The uterine and spermathecal rudiments. $\times 200$.

Fig. 33.—The uterine pore. $\times 200$.

Fig. 34.—The spermathecal pore or gonopore and the genital appendages. $\times 200$.

Fig. 35.—The Ovipositor in an Older Pupa. $\times 200$.

Figs. 36–43.—Histology.

Fig. 36.—Ovariole. $\times 250$.

Fig. 37.—Oviduct. $\times 250$.

Fig. 38.—Uterus. $\times 300$.

Fig. 39.—Uterus and dorsal sac (Spermatheca). $\times 200$.

Fig. 40.—Accessory gland. $\times 290$.

Fig. 41.—Spermatheca proper. $\times 380$.

Fig. 42.—Chitinous rod. $\times 280$.

Fig. 43.—Chitinized cylinder enclosing rectum and uterus. $\times 330$.

Figs. 44–8.—*Gastroidea polygoni* L. The Female.

Figs. 44–7.—Transverse Sections through a Young Pupa.
Anterior to Posterior.

Fig. 44.—Anterior forking of uterine rudiment. $\times 200$.

Fig. 45.—Uterine and spermathecal rudiments. $\times 200$.

Fig. 46.—Uterine pore. $\times 200$.

Fig. 47.—Spermathecal pore or gonopore and genital appendages. $\times 200$.

Fig. 48.—Transverse Sections through a Mature Pupa.

Fig. 48.—Uterus and dorsal sac (Spermatheca). $\times 200$.

PLATE 10.

Figs. 49–54.—*Gastroidea polygoni* L. The Female.

Fig. 49.—Transverse section through a mature pupa: gonopore and genital appendages. $\times 260$.

Figs. 50–4.—Histology.

Fig. 50.—Ovariole and oviduct. $\times 280$.

Fig. 51.—End of oviduct in young pupa. $\times 330$.

Fig. 52.—End of oviduct and lateral uterus in an old pupa. $\times 350$.

Fig. 53.—Accessory gland. $\times 550$.

Fig. 54.—Spermatheca proper. $\times 550$.

Figs. 55–60.—*Anthonomus pomorum* L. The Female.

Figs. 55–6.—Transverse Sections through the Eighth and Ninth Segments in the Pre-pupa.

Fig. 55.—Eighth sternite and uterine rudiment. $\times 160$.

Fig. 56.—Ninth sternite, spermatheca rudiment, and genital appendages. $\times 160$.

**Figs. 57-9.—Transverse Sections through a Mature Pupa.
Anterior to Posterior.**

Fig. 57.—Uterus and dorsal sac (Spermatheca). $\times 170$.

Fig. 58.—Uterus and chitinous cylinder. $\times 170$.

Fig. 59.—Gonopore and genital appendages. $\times 170$.

Fig. 60.—Ovarian Tubes. $\times 400$.

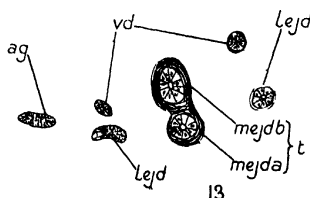
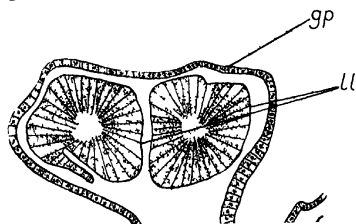
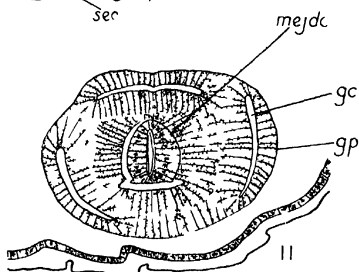
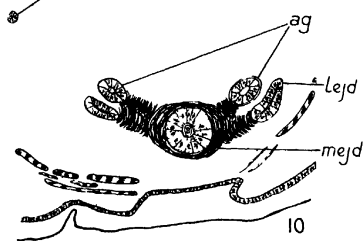
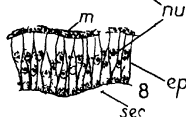
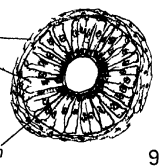
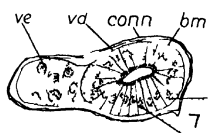
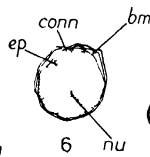
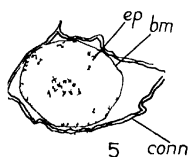
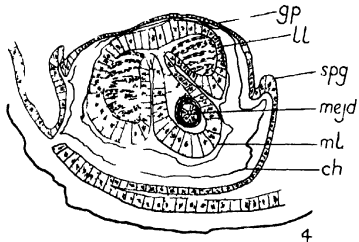
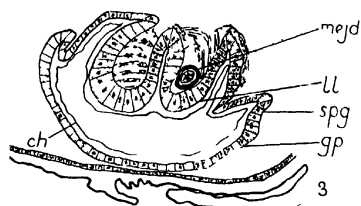
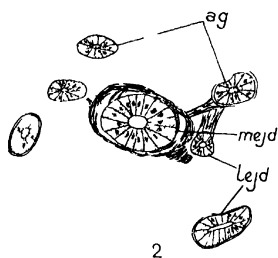
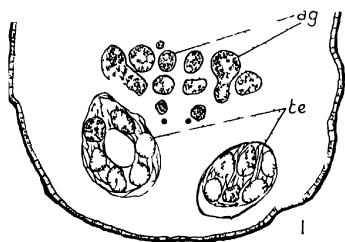
Figs. 61-4.—*Rhagium bifasciatum* F. The Female.

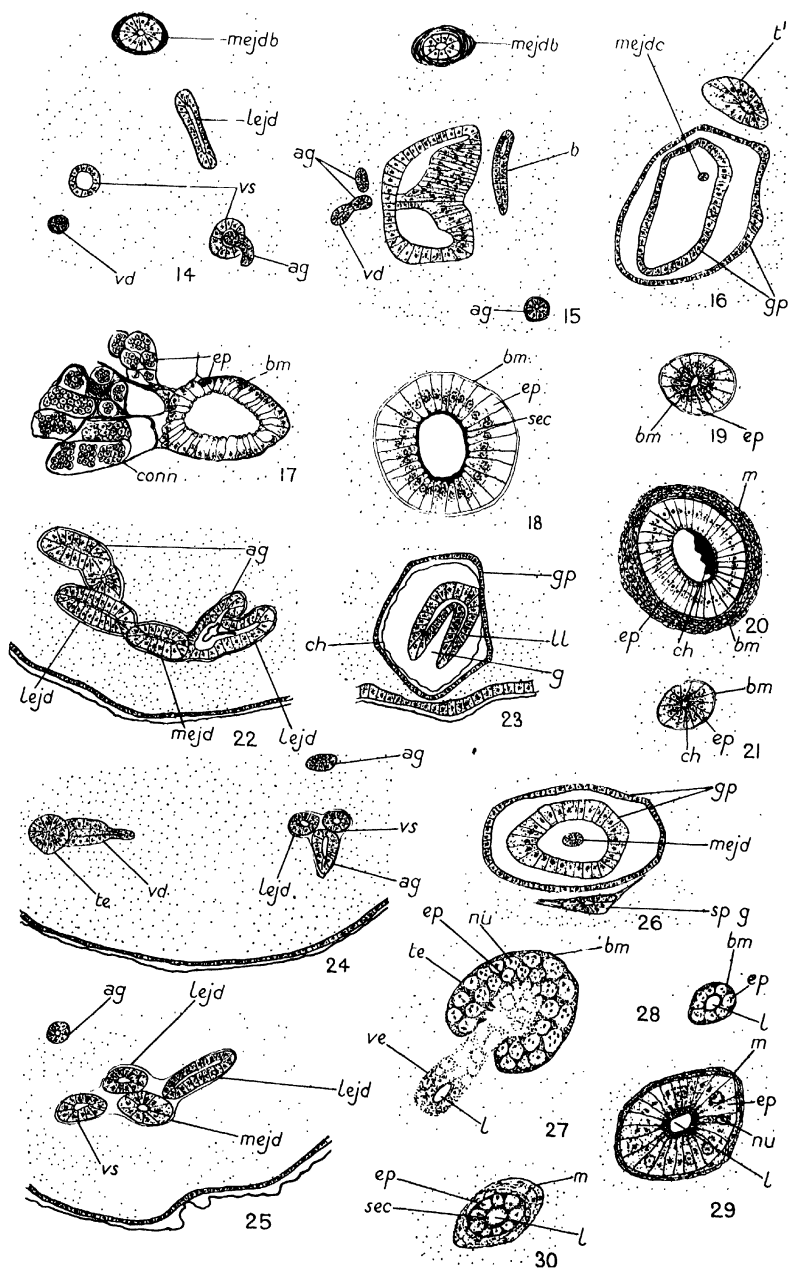
Fig. 61.—Uterus and dorsal sac (Spermatheca) in a mature pupa. $\times 130$.

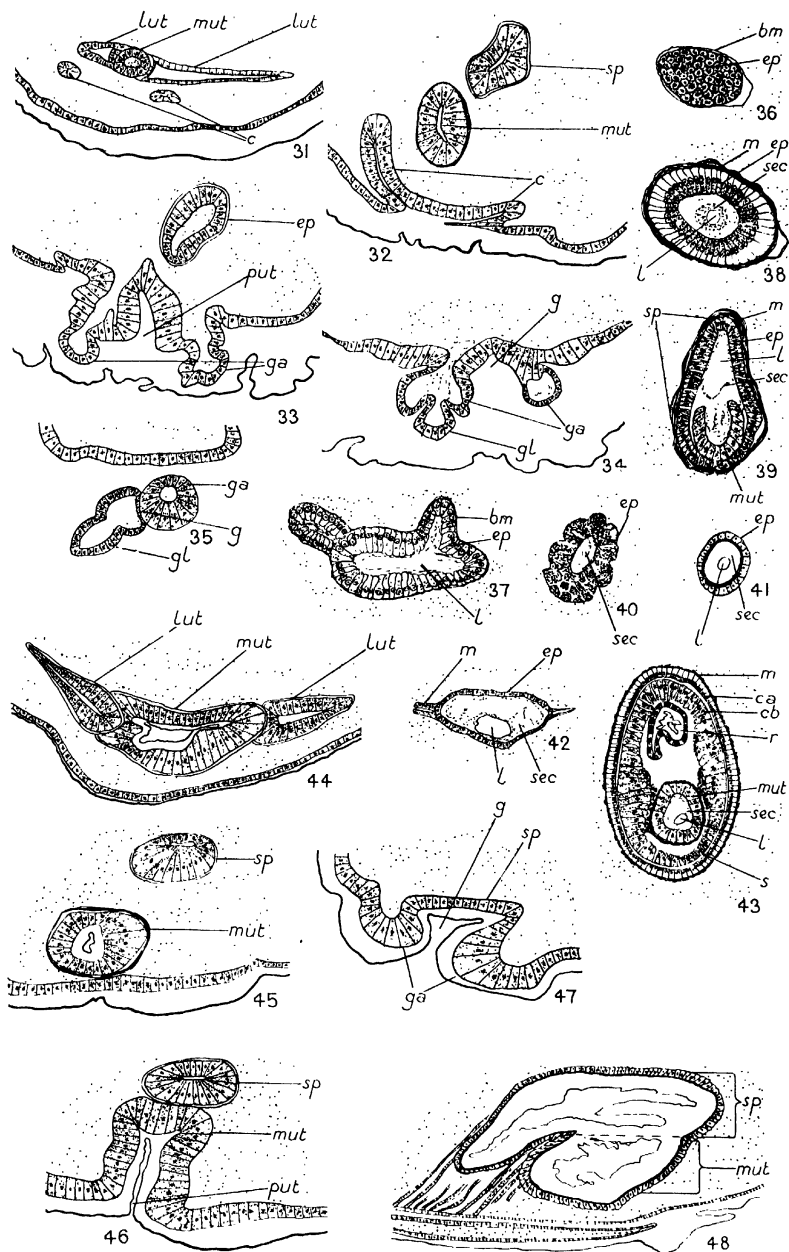
Fig. 62.—Uterus, rectum, and chitinous cylinder. $\times 130$.

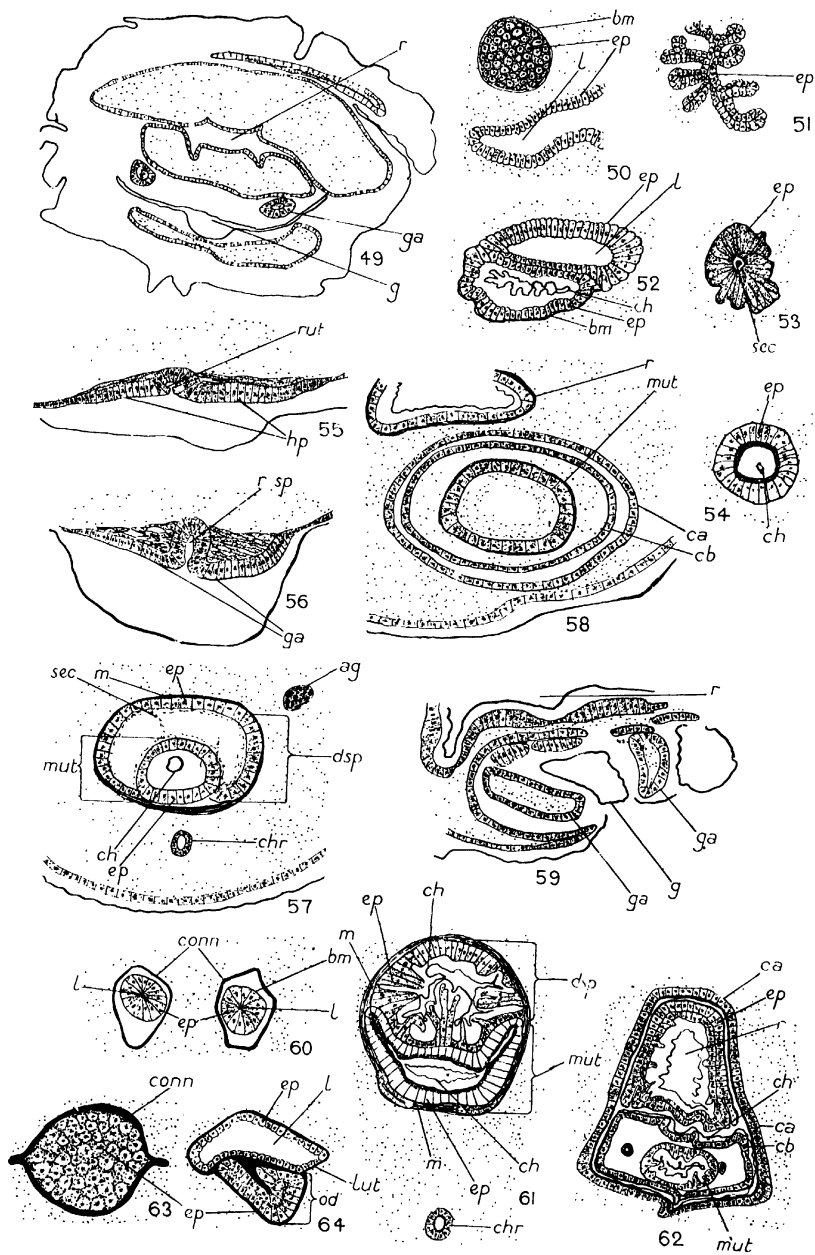
Fig. 63.—Ovariole. $\times 150$.

Fig. 64.—Oviduct and lateral uterus. $\times 150$.









On the Function of the so-called 'Rectal Glands' of Insects.

By

V. B. Wigglesworth, M.A., M.D.,

(From the London School of Hygiene and Tropical Medicine.)

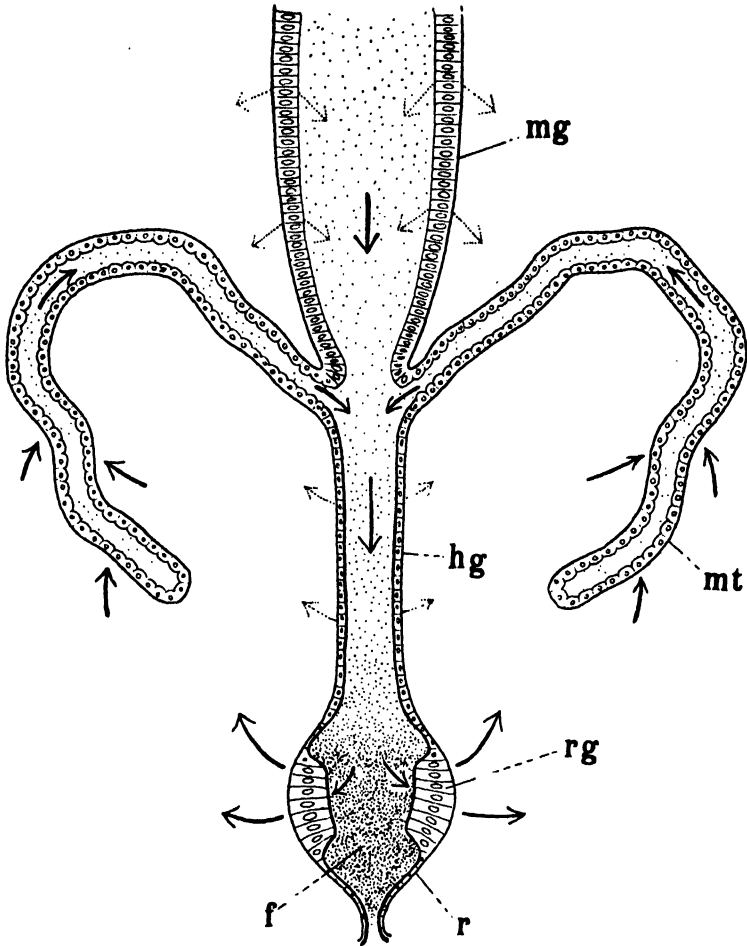
With 2 Text-figures.

It is a characteristic of those animals, such as birds and reptiles, which eliminate their nitrogen in the form of uric acid, that they show an active reabsorption of water from the excrement in the cloaca; so that there is a continuous circulation through the excretory system, enabling the animal to accomplish its excretion with a minimal supply of fluid. In the large bowel of mammals, also, the water secreted in the digestive juices is reabsorbed, and the faeces rendered more or less dry before they leave the body.

It has recently been shown (Wigglesworth, 1931, *b*) that a similar circulation of water takes place in the blood-sucking bug *Rhodnius prolixus*. In this insect much of the reabsorption occurs in the lower segments of the Malpighian tubes, but it is highly probable that some takes place also in the rectum; and on the basis of these observations it was suggested that reabsorption of water might prove to be the function of the so-called 'rectal glands', which would thus play an essential part in the all-important process of water-conservation in insects.¹ It is the object of the present paper to examine this hypothesis.

The various other hypotheses which have been advanced from time to time will be considered later; but some observations on a number of insects, made in the light of the notion here put forward, will first be described.

¹ A similar process has been suggested by Bahl (1919) for the conservation of water by certain earthworms of dry climates, in which the nephridia open into the intestine.



TEXT-FIG. 1.

Diagram of suggested course of water-circulation in alimentary and excretory systems of an insect. *mg*, mid-gut; *mt*, Malpighian tubes; *hg*, hind-gut; *r*, rectum; *rg*, rectal glands; *f*, faeces, often almost dry. Fluid is probably both secreted and absorbed in the mid-gut. It is absorbed in the hind-gut, especially in the rectum, and returned to the gut by the Malpighian tubes.

THYSANURA.

Almost all the uric acid produced by *Lepisma saccharina* is deposited in urate cells interspersed with the other cells of the

fat-body; and the Malpighian tubes discharge a clear fluid free from solid matter. This secretion is added to the fluid contents from the mid-gut and passed backwards in the hind-gut.

The hind-gut consists of two main segments: a long thin region with comparatively small epithelial cells, and a thick, very elongated rectum, the walls of which are composed of large columnar cells arranged in six longitudinal folds.

The fluid contents of the hind-gut are held up for a comparatively long time in the rectum, and here the fluid is gradually extracted from them until they are discharged as elongated pieces of dark excrement which are almost dry. It is evident that the water is absorbed by the rectal epithelium.

DERMAPTERA.

The excrement of the earwig (*Forficula auricularia*) is in the form of dry black pellets enclosed in the remnants of the peritrophic membrane. It contains little uric acid, for the bulk of this substance is accumulated in solid form in the urate cells scattered throughout the fat-body.

The hind-gut consists of four segments: (i) a region with powerful muscular walls and low epithelium; (ii) a region with well-developed cubical or columnar epithelium; (iii) a region with low epithelium; and (iv) the rectal sac, a thin-walled chamber with six prominent rectal glands (see Bordas, 1898).

The Malpighian tubes contain only fluid. In its anterior part the hind-gut contains a thick fluid with solid matter in suspension; this gradually becomes solid as it passes backwards, until, in the rectum, it is converted into the almost dry pellet that is discharged.

Since the secretion of the Malpighian tubes is fluid, and the contents of the anterior region of the hind-gut are fluid, it is evident that water is absorbed. The cubical epithelium of the middle region of the hind-gut doubtless aids this process, but the final desiccation of the faeces must be effected in the rectum, probably by the rectal glands.

ORTHOPTERA.

Conditions in *Blattella germanica* are almost identical with those in *Forficula*. Most of the uric acid is deposited in urate cells. The Malpighian tubes (as noted also by Cuénot, 1895) do not contain solid uric acid. The hind-gut consists of the same series of segments as in *Forficula*. In front it receives the fluid secretion of the Malpighian tubes and the fluid contents of the mid-gut. As these pass backwards they gradually become drier, until, in the rectum, they are converted into a dry, grey-brown pellet containing a trace of uric acid. The final desiccation must again be attributed to the rectal glands.

NEUROPTERA.

Most of the uric acid in the imago of *Chrysopa perla* is accumulated in the fat-body and hypodermal urate cells. There is no solid matter in the secretion of the Malpighian tubes. The hind-gut, which has been fully described by McDunnough (1909) consists of four segments: (i) a muscular region with low epithelium and a cuticle bearing small spines; (ii) a chamber with six bands of rather large epithelial cells and smooth cuticle; (iii) a second muscular region with small epithelial cells; and (iv) the rectum, with six button-like rectal glands.

The contents are fluid at the anterior end of the hind-gut. They become thicker in the middle chamber (ii); and in the rectum are converted into the moist but solid and compact black faecal masses. Thus, fluid is obviously absorbed in the hind-gut, especially in the rectum.

The excreta usually contain only a trace of uric acid, as demonstrated by Folin's test; but, sometimes, small opaque granules, probably of uric acid, may be seen over the outer surface of the peritrophic membrane in the posterior region of the hind-gut. This provides additional evidence for the absorption of water.

COLEOPTERA.

The excrement of the mealworm (*Tenebrio molitor*) consists of a bone-dry powder which, in the fasting insect, may con-

tain over 50 per cent. of uric acid (K. Mellanby, unpublished observations) in a finely granular amorphous form.

The hind-gut is composed of three segments: (i) a narrow anterior segment with cubical epithelium; (ii) an elongated rectum with six broad bands of high columnar epithelium; and (iii) a muscular anal canal with very low epithelium. The chitinous intima is relatively thin in the first two segments; very thick in the third segment. The second segment, the rectum, is surrounded by a delicate sheath which binds to its outer surface the convoluted upper portions of the Malpighian tubes. At the anterior end of the rectum, these parts of the Malpighian tubes, which are colourless, communicate by way of thin ducts with the deeply pigmented parts of the tubes which lie free in the body-cavity. Neither part of the Malpighian tube contains any solid matter.

The contents of the hind-gut are semi-fluid at the anterior end, but in the rectum they become quite dry, and the amorphous uric acid separates out over the surface of the food residue.

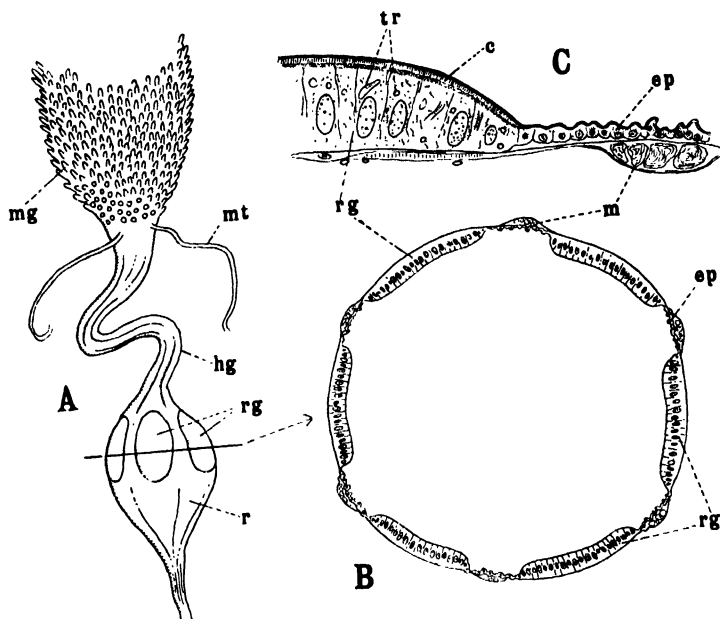
Most of these observations have already been recorded by Frenzel (1882), and there can be no doubt that Frenzel was right in concluding that water is absorbed by the rectal epithelium of these larvae.

In the adult of *Tenebrio molitor*, the conditions are the same in all essentials; though the histology of the rectal epithelium shows some interesting complications.

It is evident that in both stages of this insect the reabsorption of water is exceedingly efficient; and this is correlated on the one hand with an exceptional development of the rectal epithelium, and on the other hand with an extreme resistance to desiccation (see Buxton, 1930).

The Carabidae are an example of Coleoptera with discrete and well-defined rectal glands. According to Borri (1925) the rectal glands of *Carabus* were figured and described by Newport in 1838. They were described independently by Bordas (1914) in *Procrustes*. For the purpose of the present paper they have been studied in *Pterostichus vulgaris*.

In this insect the hind-gut (text-fig. 2) consists of a relatively narrow segment with well-developed columnar epithelium thrown into six longitudinal folds, and a capacious rectal sac separated by a sphincter. The walls of this sac are thin and the epithelial



TEXT-FIG. 2.

Pterostichus vulgaris. A, posterior region of alimentary canal; B, transverse section of rectum; c, detail of wall of rectum and rectal gland. c, cuticle; ep, epithelium of rectum; hg, hind-gut; m, longitudinal muscle bands; mg, mid-gut; mt, Malpighian tubes; r, rectum; rg, rectal gland; tr, intracellular endings of tracheae.

cells are greatly reduced, except at the anterior end, where there are six conspicuous rectal glands, oval in outline and composed of large columnar cells.

The contents of the anterior segment of the hind-gut are fluid, and the Malpighian tubes discharge a fluid secretion with solid granules of uric acid in suspension. The residue is still fluid when it reaches the rectum; and here it accumulates until the rectal sac is enormously distended. Water is gradually extracted

from the mass, and when it is finally discharged it is in the form of a large dry cylindrical pellet which varies in colour, with the nature of the food, from pale brown to black.

The final drying of the excrement is undoubtedly effected in the rectum, probably by the rectal glands.

LEPIDOPTERA.

The larva of *Borkhausenia pseudospretella* (Oecophoridae), one of the clothes moths, feeds on very dry material and is extremely resistant to desiccation.

The excrement consists of dry pellets, very rich in uric acid. The hind-gut is made up of the same sequence of chambers as described by Henson (1931) in *Vanessa*: (i) a funnel-shaped segment (ileum) with thin cuticle and small epithelial cells; (ii) an anterior sphincter with powerful muscles, small epithelial cells, and rather thick cuticle; (iii) a wide chamber (colon) with weak musculature and thin cuticle but large epithelial cells; (iv) a posterior sphincter similar to (iii) but with still thicker cuticle; (v) the rectum with thin cuticle but very large epithelial cells. Around the rectum is an annular chamber bounded externally by a thin membrane with muscle-fibres on its outer walls. The clear upper segments of the Malpighian tubes lie coiled within this chamber, closely investing the rectum. They communicate by way of thin ducts with the lower segments of the tubes which lie free in the body-cavity. These are stuffed with solid granules of uric acid in the form of minute spheres with radial striation.

These spheres, suspended in fluid, are discharged into the hind-gut and added to the semi-fluid contents from the mid-gut. As these contents pass back they gradually become drier. The drying is partially effected in the first chamber (colon), but the final desiccation and conversion into solid pellets takes place in the rectum.

The same process was suggested by Verson (1905) in the case of the silkworm, from considerations of structure, and was clearly demonstrated by Metalnikov (1908) in the larva of *Galleria*.

In the imago of *Borkhausenia pseudospretella* the meconium seems to be retained indefinitely; and if an insect which has been flying for some days is dissected, the colon is always found to be distended with a creamy mass of uric acid in a little deep yellow fluid.

The distal portions of the Malpighian tubes contain only a clear fluid. In the lower (proximal) parts, the lumen is filled with uratic spheres. The hind-gut consists of a narrow elongated segment with thin walls, a dilated chamber, usually termed colon, and a muscular canal termed the rectum. The walls of the colon bear numerous 'rectal glands', groups of three or four very large cells, each group forming a fungiform projection into the lumen, as figured by Bordas (1920).

The uratic spheres from the Malpighian tubes, suspended in fluid, are discharged into the hind-gut, which contains a colourless fluid with these white granules in suspension. The contents of the narrow segment of the hind-gut do not mix freely with those of the colon. In the colon there is relatively much more solid material, the uratic spheres tend to be larger, and the fluid in which they are suspended is a deep yellow.

These facts strongly suggest that reabsorption of water is taking place in the colon; and if so, this is probably effected by the rectal glands, for the epithelium of the intervening region is very much reduced.

DIPTERA.

The observations described above on the imago of *Borkhausenia pseudospretella* suggest that the rectal glands play an important part in reabsorbing water during pupal development; for it is obvious that it will be at this time, when the organism is cut off from external supplies of fluid, that the circulation of water through its excretory system will be most necessary. According to this conception, the function of the rectal glands during pupal life would be closely comparable with that of the cloaca and allantois in the developing chick.

This idea was next tested on *Lucilia sericata*. If this insect is dissected in the late stages of pupal development, but

before the cuticle and setae of the adult have begun to darken. the internal organs are found to be fully developed. The fat-body contains much solid uric acid (see Pérez, 1910) but the Malpighian tubes are clear and translucent. The long narrow hind-gut and the rectal pouch are empty.

Later, spherical granules of uric acid begin to appear in the Malpighian tubes, and the hind-gut and rectum become greatly distended with fluid.¹ As development proceeds, this fluid comes to contain uric acid spheres; and these increase in number until, shortly before emergence, the rectum is filled with a semi-solid mass of uric acid in a small quantity of deep yellow fluid; the rectal contents being separated from the rest of the hind-gut by a valve, obviating any reflux.

It is difficult to account for these observations without supposing a continuous reabsorption of water from the rectum; and such absorption must be ascribed to the rectal glands.

It is not easy to get satisfactory evidence of reabsorption in the adult after emergence. These flies lose fluid rapidly and do not survive for more than a day or two unless given plenty of food and water. But one observation suggests that such reabsorption may occur. Thus, it was noted that those flies which evacuated the fluid from the rectum died the soonest. Now were this fluid no longer available to the insect; in other words, were reabsorption not occurring, its loss should make no difference to the time of survival. It is interesting to compare this state of affairs with that described by Sweet (1907) in certain Australian frogs which, during aestivation, store water in the urinary bladder, and reabsorb it into the vascular system as it is required for excretory purposes.

Observations on similar lines were made on the mosquito, *Aedes* (*Stegomyia*) *argenteus*. The Malpighian tubes and rectum can be seen in the living pupa almost up to the time of emergence. Neither contains any solid uric acid, though in the later stages of development the rectum contains a certain amount of yellow fluid.

¹ When the rectum is in this distended state, the significance of the ring of thickened intima, at the base of each papilla, in preventing evagination of the epithelium, is readily apparent (cf. Cognetti de Martius, 1924).

The changes in the adult of *Aedes argenteus* after a feed of blood show that there is certainly a reabsorption of water in the hind-gut, and much of this probably takes place in the rectum. For the first day or two after feeding, the ingested blood is usually confined to the mid-gut, and the hind-gut contains only the products of the Malpighian tubes. During the first hour or so, a clear watery fluid is excreted; but when the discharge of this has ceased, nothing more is passed for about twelve hours. The excreta then consist of semi-solid masses of white or yellowish uric acid.

If the insect is dissected at about three or four hours after feeding, the rectum is found distended with clear fluid with some relatively large granules of uric acid in suspension. The lower segments of the Malpighian tubes contain a little finely granular uric acid. Thenceforward, the contents of the rectum become more and more solid with spherical masses and concretions of uric acid, and the fluid in which they lie becomes deep yellow. The uric acid in the lumen of the Malpighian tubes also increases in amount, but is always made up of minute granules.

These observations agree with those made on *Rhodnius prolixus* (Wigglesworth, 1931, *b*) where the deepening colour of the urine runs parallel with an increase in osmotic pressure and a deposition of uric acid, which are almost certainly due to reabsorption. In *Aedes argenteus* the narrow part of the hind-gut is bounded by cubical cells with a definite striated border beneath the chitinous cuticle, and it is difficult to decide the relative parts played by this epithelium and the rectal glands in the absorption of water; but the progressive increase in the solid concretions of uric acid in the rectum suggests that much of the absorption is occurring there, and is therefore to be attributed to the rectal glands.

HYMENOPTERA.

Particular interest attaches to the rectal glands of the Hymenoptera because it was in the honey-bee (*Apis mellifica*) that these organs were first observed by Swammerdam

in 1752. The hind-gut of the bee is fully described by Pavlovsky and Zarin (1922). It consists of a small intestine lined by cubical cells with a striated border and rather thick cuticle, and a distensible, pyriform rectum, in which the epithelium is greatly reduced, save where it forms the six elongated rectal glands.

It has not been possible to obtain evidence of water absorption by the rectal glands in ordinary foraging bees, because in these, as observed by Petersen (1912), the rectum always contains a clear or cloudy fluid, and the insects die within twenty-four hours of being taken. But through the kindness of Mr. D. Morland of the Rothamsted Experimental Station, I have been able to dissect nurse-bees from an observation hive in which the bees were marked according to their ages. This material has yielded good evidence for the reabsorption of water.

As noted by Petersen (1912), at the time of emerging from the cell, the rectum of the bee is distended with a clear liquid. The urate cells, loaded with solid uric acid, are conspicuous throughout the fat-body. The Malpighian tubes contain a clear fluid.

In the early days after hatching the young nurse-bees ingest large quantities of pollen; and if they are dissected when three or four days old, the rectum still shows the same degree of distension as before, but now contains a creamy yellow fluid made up of pollen grains and amorphous granules of uric acid. The urate cells are no longer apparent; but the Malpighian tubes still contain clear fluid. The uric acid has evidently been transferred from the urate cells to the rectum, where it is present in solid form.

In nurse-bees about ten days old the rectum shows the same degree of distension, but is now filled with a semi-solid mass of pollen grains and uric acid. Although, in the bees dissected, the rectal contents could scarcely be described as brick-like masses ('Ziegelähnlichen Massen')—the term used by Petersen (1912)—it was certain that much of the water had been removed. Now it is generally accepted that worker bees do not defaecate until they fly. So that it is evident that the water must have been absorbed from the rectum; and the most probable site of absorption is the rectal glands.

In the older nurse-bees the rectum becomes more and more distended, and the contents often become very fluid again; until, in bees three weeks or a month old, which had evidently started foraging and had evacuated their initial excrement, the rectum contained a clear fluid with only a little opaque matter in suspension.

The reabsorption of water from the excreta is probably an important function during the prolonged retention of the initial excrement. There is an even more prolonged retention in winter bees, which do not defaecate throughout the hibernation period; and this has already led Pavlovsky and Zarin (1922) to suggest that at this time the rectal glands must be absorbing something (they do not specify water) from the rectal contents.

SIPHONAPTERA.

In the larvae of *Ceratophyllus fasciatus* and *Xenopsylla cheopis* the elongated hind-gut is made up of a long narrow segment with relatively low epithelium, and a thickened rectum with very conspicuous columnar cells richly supplied with tracheae. (The hind-gut of *Ctenocephalus* larva is described by Harms (1912).) Towards the hind end of the narrow segment is a more dilated chamber with somewhat larger epithelium than the remainder of this region.

The secretion of the Malpighian tubes is fluid, and the contents of the hind-gut are quite fluid at its commencement. This fluid is retained for some time in the dilated chamber of the hind-gut, and apparently becomes more concentrated. It is retained for a further period in the rectum, where more concentration takes place (these changes can be observed through the cuticle of the living larva), and is finally discharged as a sticky semi-solid drop.

It is clear that fluid is absorbed in the hind-gut and rectum of the flea larva; but a considerable amount of water is lost with the faeces, and this loss, combined with the restless activity of the larvae, probably accounts for their poor resistance to desiccation (K. Mellanby, unpublished work).

In the adult flea the hind-gut consists of a long narrow segment and a distensible rectum, separated by a sphincter. The

rectum contains six rectal glands of the type occurring in the Diptera (see Lass, 1904).

The changes taking place inside the flea after a small meal of blood can be observed by transmitted light in the living insect. The blood is confined to the mid-gut. Fluid is rapidly absorbed from it, and excreted by the Malpighian tubes until the rectum is distended with a clear watery fluid. During the next day or two the blood in the mid-gut is digested, leaving only a residue of haematin; but there may be no apparent change in the rectum. Now it is certain that the Malpighian tubes must be excreting fluid throughout this period, for they contain no solid matter in the lumen. It is therefore probable that there is a simultaneous absorption of water from the rectum.

Further evidence of reabsorption is afforded, as in *Lucilia*, by the fact that the flea remains alive and active so long as the rectum is filled with fluid, but when this is evacuated the flea has a shrunken appearance and soon becomes moribund and dies. This suggests that the fluid in the rectum is of value to the insect, that is, that it is being reabsorbed into the body.

ANOPLERA.

The hind-gut of the human louse, *Pediculus humanus*, is described in detail by Sikora (1916). It consists of three parts: a thin segment with low epithelial cells and fairly well-developed musculature; a small dilatation surrounded by six conspicuous rectal glands; and a rather long muscular anal canal with thick cuticle and very low epithelium.

In the early stages of digestion the louse is apt to allow a little blood to escape from the mid-gut into the hind-gut, and it will often pass fluid drops of undigested blood. But in the later stages of digestion, the excreta are retained much longer in the hind-gut. They are quite fluid at the anterior end (as can be seen by transmitted light in the living insect), but they are held up in the little sac surrounded by the rectal glands, and are here converted into an almost dry pellet that is black or grey, depending on the relative amounts of haematin and uric acid that it contains.

The rectal glands are clearly concerned in removing water

from the faeces; and the significance of this function is very strikingly demonstrated when, as often happens, the blood is confined to the mid-gut, and the hind-gut contains only the products of the Malpighian tubes. The tubes secrete a clear watery fluid; but the absorption of water leads to a gradual accumulation of white granules of uric acid in the rectum, and these are finally discharged as a soft white pellet. The circulation of water through the rectal glands and the Malpighian tubes is as evident here as in the fasting mealworm.

DISCUSSION.¹

It is frequently stated (see Tonkov, 1923; Borri, 1925) that the irregular occurrence of the rectal glands adds to the difficulty of determining their function. Thus, they are said to be absent from nearly all larvae, most Coleoptera, and all Hemiptera.² But, as was pointed out by Chun (1876), wherever discrete rectal glands are absent, the entire rectum is lined by a uniform columnar or cubical epithelium of the same type as that composing the rectal glands; whereas, when rectal glands are present, the intervening epithelium is more or less vestigial. It is therefore reasonable to suppose that this uniform epithelium and the rectal glands subserve a common function. This was the opinion of Chun, and it has been tacitly accepted in the present paper.

All stages between a uniform epithelium and discrete rectal glands occur. For example, the larva of the mealworm is usually said to be devoid of rectal glands. But we have seen that the epithelium of the rectum is arranged in six longitudinal bands with reduced cells between. The difference between this arrangement and that in the Orthoptera is one of degree only.

The question why these epithelial cells should be arranged in specialized groups (rectal glands) in some insects, and evenly distributed in others, was considered by Chun, who concluded

¹ A full historical review of the earlier observations on the rectal glands of insects has been published by Borri (1925).

² Poisson (1924) has described localized rectal glands in the aquatic Hemiptera, and I have observed them in the Reduviid *Rhodnius prolixus* (Wigglesworth, 1931*b*).

that the purpose of the former arrangement was to facilitate changes in volume of the rectum and, consequently, to permit a more prolonged retention of the excreta.

As to the function which the epithelium subserves, Chun supposed it to be secretion; but since all observers have agreed that no digestive enzymes are produced in the rectum, and that the excretory products are derived from the Malpighian tubes,¹ there has been no satisfactory suggestion as to the possible nature of this secretion. For the idea of Petrunkevitch (1899) that they secrete a substance which facilitates defaecation is not applicable to the majority of insects, which produce moist excrement, and in any case is unsupported by histological or other evidence; and the opinion of Sayce (1897) that they are mucous glands is certainly incorrect. Abbott (1926) observed a granular zone beneath the cuticle of the rectal glands in *Periplaneta*, and therefore supposed them to be secretory. But this appearance might equally well be associated with absorption. Trappmann (1923), on similar grounds, postulated secretion in the rectal glands of *Apis*; but neither Snodgrass (1925) nor Pavlovsky and Zarin (1922) obtained any evidence of secretory changes.

If the epithelium is not secreting it is probably absorbing. Berlese (1909) believed that the rectal glands absorb the final products of digestion. Pavlovsky and Zarin (1922), writing of the hibernating bee, state that 'we may speak of the absorptive role of the rectal glands, which appears to be correct a priori, on account of the long period during which the faeces remain in the rectum'. Frenzel (1882) demonstrated the absorption of water in the rectum of the mealworm and considered it probable that other substances also might be absorbed; Verson (1905) put forward the same view in the case of the silkworm; and Metalnikov (1908) has clearly shown that water at least is absorbed in the hind-gut of *Galleria* larvae.

In the present paper much evidence has been presented for the view that the main function of the rectal epithelium is to

¹ The heterodox views of Lowne (1893), that the rectal glands of *Calliphora* secrete the solid uric acid which accumulates in the rectum during pupal life, are due to an error of observation.

absorb water. This function is more obvious in some insects than in others; but nothing incompatible with it has been observed. It is a function of first-rate importance; for the conservation of water is one of the chief difficulties with which terrestrial insects have to contend. The organs which perform it have to do very active work against the osmotic pressure of the excreta; it is not surprising, therefore, that they should be made up of large conspicuous cells with a rich supply of tracheae.

There is no difficulty in accepting the passage of water through the chitinous intima of the hind-gut; for it has been shown repeatedly (Gorka, 1914; Abbott, 1926; Eidmann, 1922) that the thin cuticle of the insect hind-gut is freely permeable to water. Whether other substances are absorbed along with the water is a problem which has not been considered in the present work. But it is worth noting that Borri (1925) observed that the rectum of *Bombyx mori* is as well supplied with rectal glands as that of the Pieridae, although the former takes no food in the adult state and, in consequence, the remainder of its alimentary system is more or less atrophic. As Borri points out, these observations do not favour the idea that the glands are absorbing nutriment; but they do not in any way prejudice the views advanced in the present paper.

Many other hypotheses have been put forward since Swammerdam discovered the rectal glands in the bee. The suggestions of Leydig that they are respiratory, and of Gegenbaur that they are vestigial remnants of rectal gills, were adequately refuted by Chun (1876). The idea of Cognetti de Martius (1924) that they are endocrine organs, is based only on the observation that they are well supplied with blood. The suggestion of Berlese (1909) that they serve to grip the peritrophic membrane and draw it slowly backwards or that of Engel (1924) that they break up this membrane before it is discharged, may perhaps be subsidiary functions of the papillae in the cyclorrhaphous Diptera, but they cannot, of course, be of general application. Nor can the suggestion that these organs serve mechanically to arrest the escape of faeces (Möbusz, 1897); and the view that they have no function but to secrete the cuticle which overlies them is untenable because this cuticle is usually much thinner

than that elsewhere in the hind-gut where the hypodermal cells are inconspicuous (see Coleoptera above).

Certain other properties of the rectal glands have been recorded which are not incompatible with the views here put forward. Kowalevsky (1889) showed that if the glands are kept on the slide for some time after dissection, they develop around them an acid zone, and he therefore supposed that they produce an acid secretion. Engel (1924), who confirmed these observations, accepts the view of Deegener (1913) that they are evidence that one function of the glands is to eliminate carbon dioxide. But any active cellular tissue treated in this way may be expected to develop acid (e.g. lactic acid), and any active organ will eliminate carbon dioxide. Pavlovsky and Zarin (1922) found that the enzyme catalase is produced in greatly increased amounts in the rectal glands of the honey-bee during hibernation, when the rectum is enormously distended with retained excrement. This is the time when, according to the reabsorption hypothesis, the glands will be most active, and the high catalase content may well be associated with this activity.

Finally, one of the stumbling-blocks in interpreting the function of the rectal glands has been their presumed homology with the rectal gills of Libellulid larvae,¹ and this, together with their rich tracheal supply, has led many authors to ascribe to them some unexplained respiratory function. Miall and Denny (1886), who agree in this with Chun (1876), state that 'it seems more probable that the respiratory appendages of the rectum of the dragon-fly larvae are special adaptations to aquatic conditions of a structure which originated in terrestrial insects, and had primarily nothing to do with respiration'. Now it was shown by Faussek (1886) that behind the rectal gills of Libellulid larvae there are six epithelial pads identical in structure with the rectal glands of Orthoptera; and Sadones (1895) shows that there is a group of similar cells (le bourrelet basal) at the base of each of the gill lamellae. The function of these cells is obscure. Deegener (1913) supposed that they eliminate carbon dioxide. But there seems to be no call for such an organ (see Wigglesworth, 1931 a), nor does their form suggest it.

¹ Sadones (1895) does not accept this homology.

It is interesting to speculate whether the function of these cells, and of many other gill-like organs of aquatic and parasitic insects, may not be to control the exchange of water between the external medium and the body fluids, and thus, like the gills of fish and the skin of Amphibia (Smith, 1930), to facilitate the process of excretion and to assist in maintaining the normal osmotic pressure of the blood (Smith, 1930). This hypothesis, which would bring these structures into line with the rectal glands of terrestrial insects (as interpreted in this paper), will be the subject of future study.

SUMMARY.

It is suggested that the rectal glands and rectal epithelium of insects reabsorb water from the excrement before it is discharged, and thus play an important part in water-conservation.

Observations on larval and adult insects belonging to all the main orders are described in support of this hypothesis.

Earlier observations and opinions on the rectal glands are discussed. Many of these are shown to agree with the theory here put forward.

In the course of this work I have received much assistance from others. I am particularly indebted to Mr. D. Morland for providing bees from his experimental hives; the developing pupae of *Lucilia* were provided by Dr. R. P. Hobson; the fleas were bred by Mr. H. S. Leeson, and the human lice by Mr. K. Mellanby; *Lepisma* and *Chrysopa* were secured by Mr. D. Gillett, and the numerous microscopic sections were cut by Mr. H. J. Sutton.

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The Origin and Development of the Anterior Lymph-Sacs in the Sea-Turtle (*Thalassochelys caretta*).

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With 6 Text-figures.

INTRODUCTION.

It requires only a hasty inspection of the literature on the lymphatic system to show the unsatisfactory state of our knowledge concerning its development. In the first place, the ontogeny and phylogeny stand in apparent conflict. There are, moreover, few features of its development upon which there is any agreement among the various investigators who have contributed to this field. As a reworking of the subject, the author was guided in choosing the turtle, not only because of the controversy over the origin of the anterior lymph-sacs, but also because in this form the embryonic anlagen of the system are easily distinguished from the blood-vessels.

In 1911 G. S. Huntington published a paper on the origin of the lymphatic system in reptilian embryos. Since his results and conclusions did not present fully all the evidence, or agree with the results of later investigators, E. L. Clark (1912), Kampmeier (1912), Stromsten (1912), Professor Stromsten suggested to the writer the advantages of repeating his work, but limiting the investigation to the turtle.

For the completion of this work. I am indebted to Professor Stromsten for his valuable suggestions and helpful criticism during the investigation.

MATERIAL AND TECHNIQUE.

The embryos of *Thalassochelys caretta* used in this work were collected by Dr. Stromsten at the Marine Laboratories of the Carnegie Institution of Washington at the Dry Tortugas,

Florida. They were fixed in a mixture of chromic acid, glacial acetic, and 40 per cent. formaldehyde. In the advanced stages the animals were narcotized before fixation in order that the delicate mesenchymal tissue might not be injured by the movements of the embryo when first placed in the fixing solution.

Throughout the preparation of sections for final mounting care was taken to prevent shrinking or tearing of the tissues. Sections were cut from 10 to 20 micra in thickness, and were stained with Delafield's haematoxylin, iron haematoxylin, or in toto with alum or borax-carmin. They were counter-stained with orange-G (slightly acidulated), eosine-aurantia-orange-G, and Mallory's connective-tissue stain.

The observations reported are based wholly upon the study of sections of embryos and camera lucida drawings, which were made serially of all stages in the region under investigation. The drawings were made on cellophane and the cellophane method of reconstruction was used.¹

It is the purpose of this paper to present results of a study of development of the lymph-sacs as well as a determination of their anlagen. By the study of serial sections it is shown that independent lymph-spaces occupy the region of the developing sac, and that these spaces by fusion aid in the formation of a plexus which later becomes transformed into the jugular sac.

OBSERVATION AND DISCUSSION.

It is evident from the literature (see bibliography) that there are three views concerning the development of the lymph-hearts:

1. The lymph-hearts arise from the veins at various centres of radiation and by continuous elongation and fusion form the hearts.
2. The lymph-hearts are derived from the embryonic venous system either by a direct transformation of certain of its channels or by the fusion of multiple derivatives which have become detached from it.

¹ Van Der Jagt, E. R., "An improvement in the technique of reconstruction work by the use of cellophane", 'Science', Dec. 1931.

3. The lymph-hearts arise by the confluence of mesenchymal spaces which invest and communicate with the capillaries.

The present observations and studies favour the third view which holds that the lymph-hearts and sacs are initiated in development by the vacuolation of the mesenchyme. They are not a product of the veins either by centrifugal growth or the fusion of detached venous elements. The endothelial lining of the lymph-sacs is a gradual differentiation from the mesenchymal tissue.

The jugular lymph-sacs of the turtle present a considerable range of variation both in the advanced structure and in the details of their development. The variations, however, are neither so great nor so numerous as in the higher forms. These variations exist not only in different embryos but even upon opposite sides of the same embryo. Besides these individual variations, one often finds certain variations due to developmental conditions. Not only do certain embryos show advanced development in certain tributaries, and retarded conditions in others, but they differ in even more minor details, as for example, in the number and arrangement of secondary tributaries and their anastomotic conditions. Although a general principle of development can be established, the actual mode of origin of certain of the veno-lymphatic anlagen of the jugular lymph-sacs cannot be stated definitely owing to the variability in development of these veno-lymphatics in conjunction with the main venous channels and their tributaries, as well as the variable manner in which they fuse together.

The active period of lymph-sac development is comparatively short. Embryos of seventeen to twenty-five days represent the important developmental stages. We may divide the history of the development of the sacs into three periods.

1. The formation of mesenchymal spaces in the anterior cardinal regions.
2. The enlargement and fusion of spaces with each other and with the venous tributaries to form the veno-lymphatics.
3. The confluence of the veno-lymphatics to form the sac,

with the transformation of the mesenchyme cells to endothelial cells.

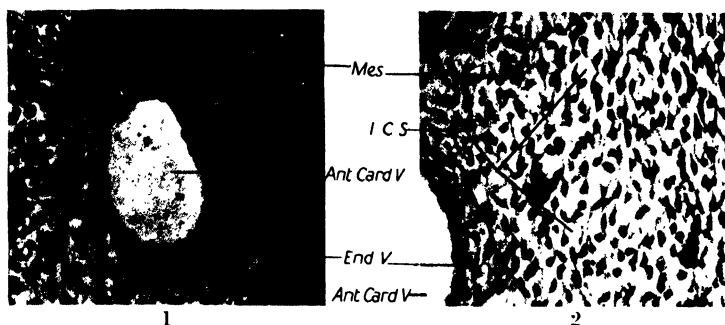
1. The Formation of Mesenchymal Spaces.

This period of development brings up the point immediately as to the source and formation of lymphatic anlagen. A careful study of the stages belonging to this developmental period points out to the observer three facts of major importance: First, the compactness of the mesenchyme at an early stage, as shown in Text-fig. 1. In the lymph-sac area of embryos at the end of the second week of development the mesenchyme is very compact, showing slight signs of vacuolation. This area is bounded by the anterior cardinal vein and its dorsal branches, the wide-meshed superficial plexus, and the ventrolateral branches of the cardinal vein. The nuclei of the mesenchyme cells in this area still show the characteristic features of mesenchyme structure as compared with the well-defined endothelial cells lining the veins in this region.

Embryos in the seventeenth day of development show marked changes. The mesenchyme in the dorsolateral region shows a loosening up of the cells. The vacuolation of the mesenchyme is forcing the cells apart. The intercellular spaces are increasing in size and number. The nuclei become widely separated and the cells are only connected by thin strands of protoplasm (Text-fig. 2).

Secondly, the formation of isolated lymphatic spaces as the result of increased vacuolation is an important step in the formation of the lymph-sacs. The rapid formation of tissue spaces cause the thin protoplasmic processes to break or disintegrate. These fine processes or strands may be seen projecting out into the lumen of the cavities thus formed (Text-fig. 3). The cavities are not lined by a definite endothelial wall but by the former mesenchyme cells of the individual spaces. The spaces are always present in the region of the developing lymph-sac and are found in direct connexion with the anterior cardinal tributaries. As these two types of anlagen unite to form the veno-lymphatics, vacuolation continues, forming again small spaces in direct connexion with the established veno-lymphatics.

This process continues even after the veno-lymphatics begin to fuse. The continual formation of these small isolated lymph-spaces in connexion with the fusing veno-lymphatics, determines the ultimate lymph-sac. The spaces occurring in the lymph-sac region are small in comparison to the lymph-spaces developed in connexion with other lymph-channels. This may



TEXT-FIG. 1: Loggerhead turtle, fourteen days; series 516, $\times 400$.

Photomicrograph of a section taken through the region of the anterior cardinal vein to show the compactness of the mesenchyme, which is comparatively free from vacuolation at this stage. The nuclei still show the characteristic feature of mesenchyme structure as compared to the well-defined endothelial cells lining the veins in this region. *Ant. Card. V.*, anterior cardinal vein; *Mes.*, mesenchyme; *End. V.*, endothelium of the vein.

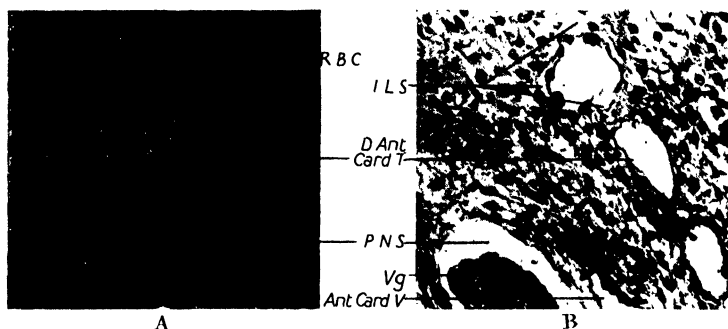
TEXT-FIG. 2: Loggerhead turtle, seventeen days; series 563, $\times 400$.

Photomicrograph of the section through the anterior cardinal vein. The mesenchyme in the dorsal and lateral region begins to appear vacuolated. This vacuolation is forcing the mesenchymal cells apart, causing an increase in size and number of intercellular spaces. The nuclei are becoming more widely separated and the cells show connexions through thin protoplasmic strands. *Ant. Card.*, anterior cardinal; *End. V.*, endothelium of vein; *I.C.S.*, intercellular spaces; *Mes.*, mesenchyme.

be due to the compactness of the mesenchyme or the more rapid reabsorption of the lymph in this region.

Thirdly, the appearance of the mesenchyme cells in this region is in marked contrast to that of the cells of an embryo in which vacuolation has not occurred in the lymph-sac region. With the appearance of the spongy mesenchyme the shape of the

cells is markedly altered. The protoplasm becomes drawn out to thin protoplasmic strands and only a limited amount of cytoplasm remains to surround the nuclei. The nuclei themselves do not present the round appearance that was observed in the compact stage, but have taken on a more flattened shape, as represented in Text-fig. 2. This change may possibly be due to the pressure of the lymph collecting in the spongy mesenchyme.



TEXT-FIG. 3, A and B.

Loggerhead turtle, seventeen and one-half days; series 8, $\times 400$.

Photomicrograph showing the two types of anlagen of the lymph-sac.

- (A) This figure shows the increased vacuolation of the mesenchyme forming the larger isolated lymph-spaces. In these larger spaces the protoplasmic strands can be seen projecting out into the lumens. Reference symbols for (A) are the same as those for (B).
 (B) Here the small isolated lymph-spaces are shown in direct connexion with the venous tributaries. These anlagen unite to form the veno-lymphatics. *Ant. Card. V.*, anterior cardinal vein; *I.L.S.*, isolated lymph-spaces; *R.B.C.*, red-blood cell; *P.N.S.*, perineural space; *Vg.*, vagus; *D. Ant. Card. T.*, dorsal anterior cardinal tributaries.

2. Enlargement and Fusion of Spaces, Investing and Communicating with the Venous Tributaries to Form the Veno-lymphatics.

In a turtle embryo of sixteen days, no lymphatics could be found in the anterior cardinal region. The partial reconstruction with the use of cellophane shows the veins along which the first lymphatic anlagen are soon to appear. The anterior cardinal vein receives segmental branches in this region. It is

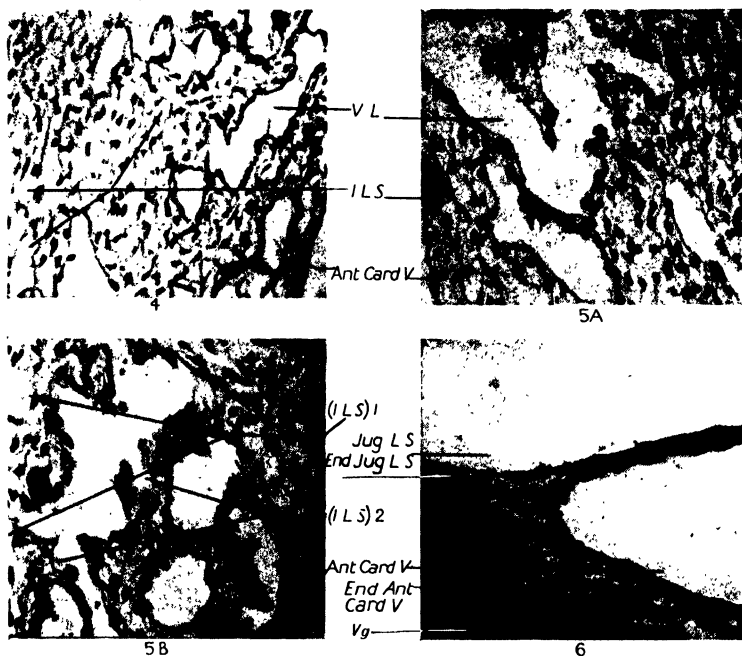
in connexion with the tributaries of these segmental branches that development of the anterior lymph-sacs is initiated.

Embryos of about twenty-days show the enlarged tributaries of the veins in the lymph-sac region, and the lymph-spaces in the direct pathway of the developing veno-lymphatics (Text-fig. 4).

The exact relationship between the venous tributaries and the spaces cannot be given at the present time. The facts remain, we have the spaces and we have the tributaries in the area occupied by the future lymph-sac. Development of the sac does not begin in any one localized place; the segmental venous tributaries increase in size all at about the same time, that is, following the vacuolation of the mesenchyme in this region.

The beginning of veno-lymphatic organization in general is indicated along the line of the primary dorsal precardinal tributaries. Here the mesenchyme begins to differentiate into a spongy, vacuolated state.

Huntington applies the term 'veno-lymphatics' to all the venous anlagen of the jugular sacs, at a time when these anlagen were filled with blood and in free communication with the venous channels. In differentiating them from early stages, he states that the only structural distinction that can be made between the venous anlagen (veno-lymphatics) of the jugular sacs and the fully formed sacs themselves, is that the former, being in communication with the veins, are filled with blood and appear to function as veins, while the latter apparently do not. During the present study it was found that there is more than this one structural characteristic to differentiate these anlagen. A careful study of the lining of these structures of Huntington during the time when they are filled with blood, reveals a marked difference in the cell structure from the cells of the fully formed lymph-sac. The cells are not the typical mesenchyme cells, neither are they the typical endothelial cells, found lining the fully developed sac, but rather represent a transitional stage of the mesenchyme cells to the endothelial cells. The blood-filled stage does not appear until several days after development of the sacs has been initiated, and not until



TEXT-FIG. 4: Loggerhead turtle, twenty days; series 237, $\times 400$.

Photomicrograph of section through anterior cardinal vein and the veno-lymphatics. The latter are continuing to increase in size by union of the isolated spaces with which they are in direct connexion. The isolated lymph-spaces are constantly being formed in the mesenchyme and are in the pathway of the enlarging veno-lymphatics. Confluence and fusion of some of the veno-lymphatics is beginning to take place as noted from the shape of the cavities. *Ant. Card. V.*, anterior cardinal vein; *V.L.*, veno-lymphatics; *I.L.S.*, isolated lymph-space.

TEXT-FIG. 5: Loggerhead turtle, twenty-one days; series 575, $\times 400$.

Photomicrographs of sections through the veno-lymphatics to show their increase in size resulting in a stage of confluence. The veno-lymphatic plexus is further condensing by coalescence and fusion of the primary dorsal and ventral divisions, with reduction of the main venous channel connexions. The area lateral to the anterior cardinal vein is well occupied by these cavities. Protoplasmic strands may be observed projecting into the lumens of the cavities. The flattened cells lining the cavities represent a transitional stage to endothelial cells. In (B) can be seen the two types of cells present in the lining of the cavities. In areas where the spaces have just been added the transitional type of

the veno-lymphatics have reached a relatively large size. Subsequent stages show a decreased blood-cell content. It was found, however, that these veno-lymphatics retain their connexions with the segmental veins. It is not until a relatively late stage of development that they lose connexion with the segmental branches and establish communication with the cardinal vein from the posterior end of the sac. Huntington has characterized the blood-filled stage, with reduction and further definition of the venous channels. One actually has the further definition of the veno-lymphatics. These are surrounded in certain areas by a network of spaces which together with a variable portion of the veno-lymphatics continues to condense into a uniform structure.

In view of the double relation which this structure sustains, on the one hand, to the embryonic venous system, and on the other hand, to the general lymphatic system with which it establishes secondary connexions, the fact must not be overlooked that this double relationship is not only revealed in an advanced stage, but that in its development both the venous tributaries and the lymph-spaces are concerned.

In using the term 'veno-lymphatic' it is not used in the same sense as it has been used in mammalian forms where it means lymphatics derived from the veins. On the contrary, it is used

mesenchyme cell can be observed, while in other areas of the same cavities typical endothelial cells can be seen. Isolated lymph-spaces are still present: (i) *I.L.S.*, isolated lymph-spaces about to be added; (ii) *I.L.S.*, isolated lymph-spaces which have been added; *V.L.*, veno-lymphatics; *Ant. Card. V.*, anterior cardinal vein; *I.L.S.*, isolated lymph-spaces.

TEXT-FIG. 6: Loggerhead turtle, about twenty-four days; series 217, $\times 320$. Photomicrograph of fully formed jugular lymph-sac. Confluence and fusion of the veno-lymphatics and spaces has continued to this time. Note how the mesenchyme cells of the veno-lymphatics have transformed into the endothelial type of cell. Reduction of the multiple early connexions between the veno-lymphatic plexus and the permanent veins has taken place. The permanent communication from the posterior region of the sac with the vein has now been established at the jugular subclavian tap. *Jug. L.S.*, jugular lymph-sac; *Ant. Card. V.*, anterior cardinal vein; *Vg.*, vagus; *End. Jug. L. Seg.* Endothelium of the jugular lymph-sac. *End. Ant. Card. V.*, endothelium anterior cardinal vein.

in the sense that the venous endothelium is only in part the source of the lining of the veno-lymphatics, and that the lymphatic endothelium arises independently of the venous endothelium by the flattening of the original mesenchyme cells. Thus it may be said that the lining of the anterior lymph-sacs is of a mixed origin, partly from the vascular endothelium and partly from the mesenchyme cells.

This raises the question that, if this be the case, why is it not possible to observe the difference in the cell structure of spaces added to those of the veins. According to the local origin theory, mesenchyme may transform into vascular tissue in practically any region of the body; and can transform into endothelium or vice versa. With this in mind, and also the fact that certain of the segmental venous tributaries atrophy while others anastomose with each other to form other veins, it appears that in certain of these tributaries the endothelial cells are reverting back to mesenchymal cells. This makes them unrecognizable from the partially transformed mesenchymal cells of the lymphatic spaces.

3. Confluence of the Veno-lymphatics to Form the Sacs, with the Transformation of the Mesenchyme Cells to Endothelial Cells.

Text-fig. 5 represents a stage of confluence of the enlarged veno-lymphatics. In this stage the veno-lymphatic plexus is further condensing by coalescence and fusion of the primary dorsal and ventral divisions, with a reduction in the main venous channel connexions. Growth of these structures has continued so that the area lateral to the anterior cardinal veins is well occupied by these cavities. Confluence and fusion of the cavities and spaces continues to the twenty-fourth day when the lymph-sacs are well formed (Text-fig. 6). Up to the twenty-fourth day protoplasmic strands may be observed projecting into the lumens of the cavities due to their enlargement and fusion. These cavities are very irregular in size and shape, but still communicate with the segmental veins. At twenty-two days there is found a further reduction of the multiple early connexions between the veno-lymphatic plexus and the per-

manent veins, and, as a result, a more complete separation of the former from the latter, with the plexuses or sac-like structures assuming a greater degree of independence. This separation and loss of early communicating channels appears to proceed from both extremities of the area toward the jugular subclavian sac where the embryonic connexions are retained longest and where the permanent adult communications between the lymphatics and the veins are established. This is in accord with Huntington and McClure's work on the mammalian lymph-sac. The cells lining the cavities are somewhat flattened representing a transitional stage to endothelial cells. These sacs do not come to lie on the muscle-plates as the lymph-hearts do, nor do they receive striated muscle in their walls in the stages studied.

The formation of the lymph-sacs shows the close connexion which these structures have or assume with the cardinal veins. From their first appearance, the cavities around which the mesenchymal cells condense and which precede the formation of the real sacs, are, in the last analysis, nothing but the fusion of spaces with tributaries of the segmental veins which have gradually grown in volume and fused together in like manner.

SUMMARY.

1. In the lymph-sac area of embryos at the end of the second week of development the mesenchyme is very compact, showing slight signs of vacuolation. The area is bounded by the anterior cardinal vein and its dorsal branches, the wide meshed superficial plexus and the ventro-lateral branches of the cardinal vein. Within this area the lymph-sac anlagen first appear, lying in the angle between the dorso-lateral branches and the cardinal vein itself.

2. The dorsal tributaries of the pre-cardinals are concerned in the development of the lymph-sacs. In the mesenchyme surrounding these tributaries isolated spaces develop in direct connexion with the veins.

3. The development of the anterior lymph-sacs in the sea-turtle is initiated by vacuolation of the mesenchyme in the region

of the cardinal tributaries during the middle part of the third week of development.

4. A series of channels is formed from the anterior cardinal tributaries and isolated spaces which in their greatest development exist as plexuses of vessels connected with the cardinal veins.

5. These channels enlarge and fuse with each other, the remnants of their walls appearing as protoplasmic fibres in the lumen, producing a system of cavities very irregular in size and shape. They inter-communicate and connect at the same time with the dorsal tributaries of the pre-cardinal veins. The mesenchyme cells surrounding these cavities are gradually transformed to the flattened endothelial cells which later line the sacs.

6. The lymph-sac is well developed at twenty-four days. Fusion and enlargement of individual cavities has continued up to this time.

7. As the development of the lymph-sacs proceeds they lose connexion with the cardinal veins except for the opening at the posterior end of the sac.

8. The development of the anterior lymph-sacs in the turtle shows two distinct anlagen, one from the mesenchyme and the other from the venous tributaries, which unite to form the veno-lymphatics. The increase in size and confluence of the veno-lymphatics determines the development of the anterior lymph-sacs.

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On the Nephridiostome of *Lumbricus*.

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With Plates 11 and 12 and 2 Text-figures.

It might be thought that little remains to be said about such a familiar object as the nephridial funnel, or nephridiostome,¹ of the earthworm, *Lumbricus*. Its structure in the adult has been studied by several eminent zoologists, among whom may be mentioned Gegenbaur and Benham, and its development described by Vejdovsky, Wilson, and others. Yet opinions still differ as to its derivation and general morphology; and the latest detailed study of the funnel by Rosen (1911) contains not only some misleading statements as to facts, but some theoretical conclusions as to the homology of its parts which seem to me quite unjustified. For, basing his view chiefly on Ed. Meyer's (1886) observation that in the Polychaete *Polymnia* the funnel and canal of the excretory organ are of separate origin (the former developing from the coelomic epithelium), and on certain doubtful observations made by Berg (1899) on the development of the nephridium of *Rhynchelmis*, Rosen concludes that the marginal cells of the lumbricid funnel are derived from the coelomic epithelium.

Already Benham (1904) had suggested that the nephridium of the Oligochaete *Haplotaxis* may be a nephromixium, owing apparently to a somewhat vague resemblance between the nephridium and the sperm-duct. Similar suggestions have

¹ Since the term nephrostome has been loosely used for many funnel-like structures, some of which are certainly not homologous with the funnel of the nephridium of *Lumbricus*, I have recently (1930) used the more definite term nephridiostome for the funnel belonging to and derived from the true nephridium.

been from time to time expressed by other authors, for instance Boveri-Boner (1920).

In a general paper (1895) I endeavoured to show that two quite different organs, the coelomoduct (derived from the wall of the coelom) and the true nephridium, may be distinguished in all groups of Coelomata; and, further, that the coelomostome or funnel of the coelomoduct, should not be confused with the opening into the coelom of the true nephridium (nephridiostome) found in Oligochaeta (and some Polychaeta). The term nephromixium was introduced by me (1900) to denote the compound organ found in certain families only of Polychaeta, formed by the grafting of a coelomostome on to a nephridium. I can see no justification for the application of this term to any organ in the Oligochaeta. Even if it could be proved that certain cells of the lumbricid funnel were derived from coelomic epithelium, this would be no good reason for using here the term nephromixium, since in the Oligochaeta coelomostomes and nephridiostomes are quite independent and may coexist in the same segments.

A renewed study of the structure and development of the funnel seemed, therefore, desirable, and indeed necessary, if a correct interpretation of its general morphology is to be reached.

The following observations were made on living and preserved nephridia of *Lumbricus terrestris* L. The best fixative is Bouin's fluid, but other familiar fixatives give good results. Sections were mostly stained in borax carmine followed by picronigrosin for general purposes; and in Mann's methyl-blue eosin, and iron-haematoxylin for special points and comparison. I have to thank Mr. G. R. de Beer for making the reconstruction in wax used for text-fig. 1, p. 168.

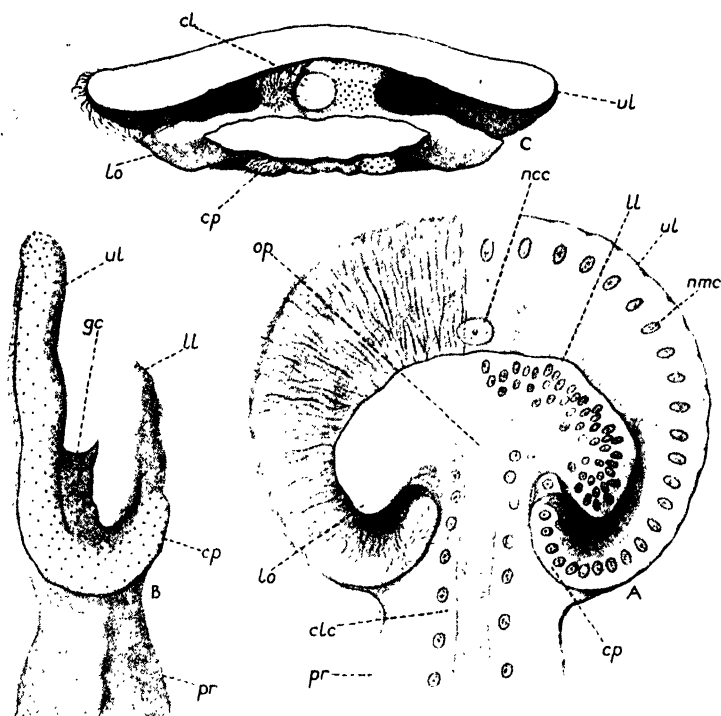
NEPHRIDIOSTOME OF THE ADULT.

The early history of our knowledge of the structure of the nephridiostome of *Lumbricus* and allied genera has been sufficiently dealt with by Benham (1891) and Rosen (1911), and need not delay us here. Of all the descriptions hitherto given of this organ that of Benham is the most correct and may be used as our starting-point. He describes the expanded upper or

dorsal lip provided with an even covering of cilia and an outer coat of coelomic epithelium. The narrow preseptal nephridial canal formed of pierced 'drain-pipe' cells, with right and left bands of cilia, on reaching the middle of the funnel opens into the coelom, its walls bending outwards and then backwards on each side. The drain-pipe cells here are continued into grooved 'gutter-cells' along which extend the ciliated bands. The latter cells, he says, join the inturned ends of the right and left horns of the crescentic row of marginal cells surrounding the dorsal lip. Thus, if I understand him rightly, these 'centripetal' marginal cells are said to meet the 'centrifugal' gutter-cells on the ventral side of the funnel, a statement which does not quite correctly represent the true state of affairs (see below and Text-fig. 2). Benham discovered that the marginal cells surround a large central cell occupying the middle region of the dorsal lip. But, though he clearly distinguishes between the mass of coelomic corpuscles on the funnel from the funnel itself, he gives no very clear description or figure of the lower lip. Moreover, his figure of the whole funnel (Pl. 23, fig. 4) does not correctly indicate the disposition of the gutter-cells. Nor does the figure since published by K. C. Schneider (1902) represent any better the true relations of gutter-cells, marginal cells, and lower lip.

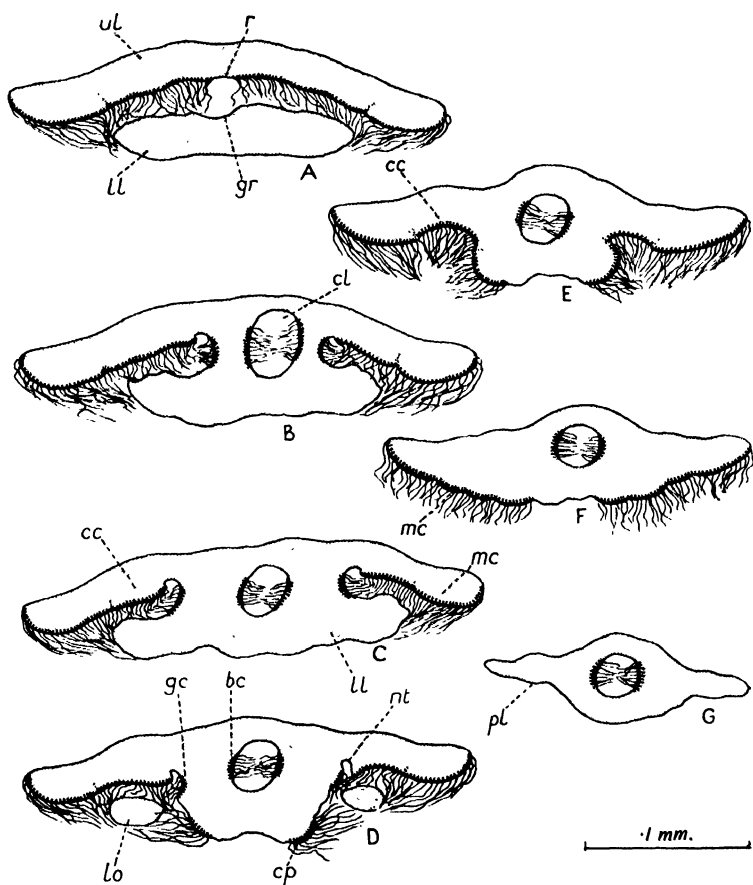
In 1911 F. Rosen brought out a detailed work on the nephridiostome of *Lumbricus*, based on the study of *Lumbricus agricola* Hoffm. On the whole he confirms Benham's description, adding certain details, some but not all of which seem to be correct. His diagram of the whole funnel (Pl. 12, fig. 9) closely resembles my own reconstruction.

Rosen describes the marginal, central, and canal cells as provided with a 'cuticula'; but I can find no such cuticle on any of these cells apart from the cell-wall. On the other hand, the closely set cilia are provided with distinct basal granules which, when stained with iron-haematoxylin for instance, form a very conspicuous marginal layer (Text-fig. 2 and figs. 7, 8, and 9, Pl. 11. The central cell is crescentic in shape, and Rosen points out correctly that where the dorsal wall of the canal meets the concavity of the central cell at the base of the dorsal lip there is a thin area with neither nucleus nor cilia. This



TEXT-FIG 1.

Diagrammatic figures of the nephridiostome of *Lumbricus terrestris* L. drawn partly from a reconstruction in wax made by Mr. G. R. de Beer, and partly from sections and living specimens. *A*, ventral view; the cilia of the upper lip, *ul*, are shown on the right, and the nuclei of upper and lower lips on the left. *B*, side view; the ciliated areas are marked with dots. *C*, anterior view looking into the mouth of the funnel; the upper and lower lips have been partly cut away; cilia are shown on the left, and ciliated areas marked with dots on the right. In *B* and *C* the space between the lateral lobe of the lower lip and the margin of the upper lip has been somewhat exaggerated to expose the 'gutter'. *cl*, canal of nephridium; *clc*, canal-cells; *cp*, centripetal marginal cells of ventral horn of horse-shoe; *gc*, gutter-cells extending to lateral margin of lip; *ll*, lower lip; *lo*, lateral lobe of lower lip; *ncc*, nucleus of central cell; *nmc*, nucleus of marginal cell; *op*, opening of canal into coelom; *pr*, preseptal region; *ul*, upper lip.



TEXT-FIG. 2.

Seven transverse sections through the nephridiostome of *Lumbricus terrestris* L. *A*, is the most anterior and corresponds to the cut surface in text-fig. 1. *C*, *G*, the most posterior, passes through the preseptal canal just behind the funnel. Drawn with the camera lucida, the basal granules of the cilia being diagrammatically shown. *bc*, lateral band of cilia in canal; *cc* central cell; *cl*, canal; *cp*, 'centripetal cells' of ventral horn of margin of upper lip; *gc*, band of cilia of gutter-cells; *gr*, groove on base of lower lip; *ll*, lower lip; *lo*, lateral lobe of lower lip; *mc*, marginal cell; *nt*, notch between central and gutter-cells; *r*, non-ciliated dorsal region at base of upper lip.

seems to be due to the fact, already mentioned, that the cilia of the canal cells are set along two longitudinal lateral bands which pass over on each side on to the everted lips of the funnel formed by the gutter-cells, and that the nuclei are also situated at the sides. The gutter-cells are of exactly the same histological structure as the canal cells, and are undoubtedly of the same nature.

Rosen describes the marginal cells of the two horns of the horse-shoe as bent round ventrally so that their ciliated surface faces outwards; but insists strongly that the gutter-cells do not meet them. According to my own observations, the gutter-cells do meet the inner ends of the marginals, not as Benham appears to think at the ends of the horns, but on the everted lateral lips of the funnel. Indeed, it is difficult to see how otherwise these lips could be completed. The band of cilia continued from the canal on to the everted lip therefore meets the ciliated area belonging to the lateral marginals, though in some specimens the band is much attenuated before the junction takes place. The continuity of the marginal ciliated area with the ciliated band of the gutter-cells is clearly shown if the figs. 8 and 9, Pl. 11, representing two consecutive sections 4μ thick are superimposed.

An important point, which seems to have escaped the notice of previous authors, concerns the finer structure of the funnel-cells. As shown in figs. 1-4, Pl. 11, the outer region of the marginal cells, that region towards the ciliated surface, is composed of dense finely granular cytoplasm in which delicate filaments can sometimes be seen running inwards from the basal granules of the cilia. In the central cell this outer layer is less dense, and appears paler in sections. But the inner region of the marginal cells, that region farthest from the ciliated surface, is of quite different structure. It appears in sections as much vacuolated with strands or trabeculae of cytoplasm of a more or less fibrous nature passing between the spaces. Into this region penetrate scattered connective tissue-cells. This characteristic structure belongs also to the central cell gutter-cells and canal cells, figs. 1-4 and 9, Pl. 11, and may be considered as strong evidence that all these cells are of the same kind and origin (see further, p. 175).

The lower or ventral lip has not been clearly described by

previous authors. Rosen's account of it is the least satisfactory part of his work. The general shape of the lower lip is seen in Text-fig. 1. It is smaller than the upper lip, has a narrow base and two backwardly directed lateral lobes which project into the spaces bounded by the upper lip, everted lateral lips and ventral horns of the marginal horse-shoe. It is not ciliated. It must be remembered that the whole of the exposed surface of the funnel and preseptal canal is covered with coelomic epithelium, as Berham showed. Along the edge of the upper lip and everted lateral lips this epithelium reaches to near the margin. The marginal cells, however, form the actual rim of the funnel along upper and lateral lips, figs. 1 and 2, Pl. 11. Much loose connective tissue lies between coelomic epithelium and canal wall in the preseptal region, and some connective tissue-cells extend into the lips. Now, if I understand him rightly, Rosen believes that the bulk of the lower lip is not truly a part of the funnel, but is formed entirely of coelomic epithelium and connective tissue. Influenced, apparently, by K. C. Schneider's description of the nephridiostome of *Eisenia rosea* (1902), he repeatedly maintains that the lower lip is without nuclei, and figures it as such (p. 169, Text-fig. 7). According, then, to Rosen's text and figures the true lower lip would be merely the short projecting edge of the terminal canal cell. For this interpretation there appears to be no justification, and we shall see when studying the development that, just as in the case of the upper lip, the coelomic epithelium covers the outer surface only of the lower lip, while its inner surface and rim are formed of cells of nephridial origin. Since these cells are not ciliated they differ considerably in shape and structure from those of the upper lip. They form an irregular more or less columnar epithelium with oval nuclei, figs. 1 3, 5-7, Pl. 11. The coelomic epithelium cells, on the other hand, consisting of flattened cells with smaller more darkly staining nuclei, can generally easily be distinguished from the nephridial epithelium, though the limit between them is often not clearly marked. The lateral margins of the lower lip are often drawn out into sharp irregular edges against which the cilia of the upper lip are closely applied, figs. 2 and 7, Pl. 11.

Attached to the coelomic epithelium of the lower lip is that peculiar mass of coelomic corpuscles or leucocytes which has so often been described. Since it forms no essential part of the funnel, and has been dealt with thoroughly by Benham, Rosen, and others, it need not now detain us. At its point of attachment the coelomic epithelium is often considerably modified and disturbed, if not actually injured; but the limit between the two can generally be made out. In well-preserved sections the corpuscles are seen to differ in shape and composition from the coelomic epithelium cells, and to stain differently, especially in iron-haematoxylin.

DEVELOPMENT OF THE NEPHRIDIOSTOME.

It is generally agreed that, in *Oligochaeta*, part if not the whole of the nephridium is derived from a large cell, the nephridioblast. In this paper we are not concerned with the first origin of this nephridioblast—whether it should be called ectodermal, mesectodermal, or mesodermal. Much controversy has taken place on this point in the past, and every view has been advocated. For our present purpose we are concerned only with the origin of the nephridiostome, and the point we wish to decide is whether it is derived from the nephridial rudiment, from the coelomic epithelium, or from both. Wilson (1899), one of the first to examine in detail the development of the nephridium in *Lumbricus*, believed that the large anterior ‘funnel cell’, which gives rise to the funnel, is of separate origin from the nephridial canal. But, Vejdovsky, our greatest authority on this question, who studied the development of nephridia from 1884 to 1892, finally came to the conclusion that the nephridioblast gives rise by division to both the canal cells and the funnel-cell. The latter pushing its way through the septum on to its anterior face, according to him, forms the funnel (1892). Similarly Berg (1888, 1890) describes both canal and funnel as derived from a single rudiment. Lehmann (1887) had previously come to the same conclusion. Later Berg (1899) studied the development of the nephridium in *Rhynchelmis*, a *Lumbriculid*, where the funnel is smaller and simpler than in

Lumbricus, having an upper lip of eight ciliated marginal cells and a lower lip of four non-ciliated cells. He concluded that, whereas the canal cells and lower lip cells are all derived from the nephridioblast, the upper lip marginal cells come from the coelomic epithelium. According to Staff (1910) on the contrary, in *Criodrilus*, a Glossoscolecoid, the funnel cell gives rise to the upper lip and canal cells to the lower lip.

From this brief review of the literature it may be said that it is now generally agreed that both the canal and the funnel (or some part of it) are developed from a single large cell, the nephridioblast, a conclusion which is borne out by what we know of the development of the nephridia in the lower *Oligochaeta* from the observations of Vejdvosky and the more recent work of A. Meyer (1929). The only point which remains undecided is whether the whole nephridiostome (excluding, of course, its external covering of coelomic epithelium and the intrusive connective tissue) arises from the nephridioblast, or whether coelomic epithelium contributes any part of the upper lip proper.

The account of my own observations may begin with the stage shown in fig. 10, Pl. 12, of a portion of a longitudinal sagittal section near the tail end of an advanced embryo. Here 'funnel-cells' appear in four consecutive segments. Each nephridial rudiment consists of a post-septal string of cells reaching obliquely backwards to the epidermis, and ending anteriorly in a large funnel-cell bulging forwards through the coelomic epithelium into the cavity of the next segment. Although the nephridial cells can be distinguished easily enough under the high power lens from surrounding tissues, the contrast in staining properties has been somewhat exaggerated in this and succeeding figures for the sake of clearness. Sections of slightly later stages show that in place of the single large funnel-cell there is a group of large cells which have every appearance of having been derived from it. This group may be called the funnel rudiment (from figs. 11-15, Pl. 12). As yet there is no lumen either in the rudiment of the canal or in the funnel region. The cells of the funnel rudiment begin to arrange themselves round the point where the mouth of the funnel will later appear, and the first indication of this opening is seen, in sections passing

through the centre of the rudiment, as a small notch between the developing upper and lower lips (figs. 16, 18, and 19, Pl. 12). This notch deepens and the lumen extends more and more into the canal region (figs. 20-9, Pl. 12). Cilia soon appear on the future upper lip. By this time the cells of the whole nephridial rudiment have increased in number by repeated division and come to surround the lumen. The canal region early becomes bent away from the surface and bulges into the coelom carrying a layer of coelomic epithelium with it.

In the development of the funnel rudiment dividing nuclei frequently are found, but it is difficult to trace the fate of individual cells. How many of these cells are derived from the original 'funnel-cell' and how many from the foremost of the canal cells I could not determine. But an unusually large nucleus is generally found in a cell at the anterior edge of that part of the rudiment which later gives rise to the lower lip, and this large cell is undoubtedly a derivative of the 'funnel-cell' (figs. 13 and 14, Pl. 12).

In these and later stages the upper and lower lips of the funnel become more and more distinctly differentiated. In the earlier stages the cells forming the funnel rudiment have larger nuclei, but differ in no essential from those of the canal rudiment; in fact canal cells and funnel-rudiment cells are in all essential respects similar. The central cell of the upper lip is not clearly distinguishable till the stage at which the lumen has been formed in the funnel (fig. 29, Pl. 12).

Figs. 30-2, Pl. 12, show three successive transverse sections through a young funnel. The upper and lower lips are now clearly developed, and the continuity of these two projecting lips at the sides is obvious.

With regard to the lower lip the figures already mentioned show that it is throughout developed from the funnel-rudiment, which itself is from the first merely the anterior part of the whole nephridial rudiment. As seen in these figures and in figs. 33 and 34, Pl. 12, of longitudinal sections of later stages, the lower lip is formed of many cells which increase in numbers to form the lower lip of the full-grown funnel. The examination of numerous intermediate stages has convinced me that these

cells do indeed form the epithelium at the edge of the lower lip of the adult worm described above, p. 171.

In the later stages the nuclei of the cells of the preseptal canal become arranged in two lateral rows, the dorsal and ventral walls being deprived of nuclei. This disposition of the nuclei extends into the base of the lips and into the lateral everted gutters; but the free edge of the lower lip and its lateral lobes are formed of an epithelium of many cells.

In the same way, my observations show that the upper lip is also formed from the funnel-rudiment, itself a portion of the whole nephridial rudiment, and derived from the 'funnel-cell', possibly also from the most anterior canal cells. Strong evidence that the marginal and central cells belong to the nephridial rudiment is afforded by the observation that the peculiar vacuolation of the basal region of the future central and marginal cells (described above in the funnel of the adult, p. 170) appears quite early, indeed, even before the lumen has begun to pierce the funnel-rudiment (figs. 16, 17, 19, 20-2, Pl. 12). Thus the entire nephridiostome (excluding, of course, the external covering of coelomic epithelium and intermediate layer of connective tissue) appears to be an integral part of the nephridial rudiment, and of any participation of coelomic epithelium cells in the formation of either upper or lower lip I can find no evidence.

This conclusion is borne out by a comparison with other related forms. As we pass from such simple forms as the Tubificidae through the Lumbriculidae to primitive Lumbricomorpha such as the Haplotaxidae (possibly ancestral to the Lumbricidae), the nephridiostome is seen to become more and more elaborated, as has been well described and figured by Y. Boveri-Boner (1920). In *Tubifex* it has only three or four nuclei, its rim has delicate cilia extending into the coelom while the upper lip is provided with a flame-like bunch of cilia working in the lumen. This 'flame', as I long ago suggested (1895, 1896), probably represents in the smaller and more primitive *Oligochaeta* the original ciliation of the protonephridial flame-cell from which the nephridiostome has doubtless been derived in phylogeny. In the more advanced *Lumbriculus*, larger

dorsal and smaller ventral lip are present. The former consists of a central and two marginal cells somewhat vacuolated; yet the inner flame-like bunch of cilia is still distinct from the peripheral cilia. *Haplotaxis gordioides*, according to Boveri-Boner, has a nephridiostome very like that of a Lumbricid. It is borne on an elongated preseptal canal, there is a large upper lip with one central and many marginal ciliated cells, and a lower lip with many nuclei. The 'flame', however, may still be distinguished on the central cell. It is but a step from this to the funnel of the earthworm where the marginals are more numerous and the ciliation of the central cell has lost its flame-like character.

Moreover, since A. Meyer (1929)¹ has recently given a detailed and careful account of the development of both canal cells and nephridiostome from the nephridioblast in *Tubifex*, thus corroborating Vejdovsky, the evidence seems to be complete that the nephridiostome is an integral part of the nephridium in Oligochaeta in general.

It follows that the attempts made by certain authors (see p. 165) to homologize the funnel of an Oligochaete with that of the nephromixium of a Polychaete must be rejected. They are perhaps due to the failure of these authors to appreciate the fact that the formation of a compound nephromixium has only been proved to occur in the Order Polychaeta, and only in certain families of that Order; that it seems very unlikely that this peculiar combination should have come about in any other

¹ With the first origin of the nephridioblast we are not concerned in this paper; but I may point out that, although A. Meyer clearly traced the development of the whole nephridium from a nephridioblast lodged among coelomic epithelium cells on the anterior surface of the septum and concluded (unlike Vejdovsky) that this cell is derived from the coelomic epithelium, he does not seem to have proved his case. The first stage he describes is already a late stage in development when the coelomic cavity is large and the septum thin. At this stage the nephridioblast, whatever its origin may be, must perforce take up a position in the coelomic epithelium. From a personal inspection of Meyer's preparations I am convinced that he is mistaken and failed to trace the origin of the nephridioblast in *Tubifex*. Furthermore, there seems to be no justification for Meyer's extraordinary theories concerning the general morphology of nephridia and genital ducts in the Annelids.

Order; and that, therefore, the view that a nephromixium has been formed in any other Order should only be accepted on the strongest evidence. Since in *Oligochaeta* nephridia and coelomoducts (genital funnels) admittedly exist separately and side by side, of all the groups of Annelids it is the one in which we should least expect to find such nephromixia.

SUMMARY.

The structure of the nephridiostome of *Lumbricus terrestris* L. is described, including the anatomical relations of canal, gutter, central, and marginal cells and their cytological characters. The extent and relation of the lower lip to other parts are also described.

An account of the development of the nephridium is given from the stage when the rudiment still bears a single large 'funnel-cell' bulging forwards through the septum into the coelom. The whole nephridiostome (excluding the covering of coelomic epithelium and the connective tissue) is shown to arise from the nephridial rudiment, wholly or partly from that part of the funnel-rudiment which is derived from the 'funnel-cell'. Upper, lateral, and lower lips are all developed from the funnel-rudiment in which the lumen becomes pierced. There is no evidence that the coelomic epithelium contributes any part of the true nephridiostome.

The view sometimes put forward that the excretory organ of *Lumbricus* is a nephromixium is not founded on sound evidence, and is opposed to the simple straightforward interpretation of its morphology which follows most naturally from the facts and a comparison with lower forms.

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EXPLANATION OF PLATES.

REFERENCE LETTERS.

bc, lateral band of cilia in nephridial canal; *bv*, blood-vessel; *bw*, body-wall; *c*, coelom; *cc*, central cell of upper lip; *cep*, coelomic epithelium; *cl*, canal of nephridium; *clc*, canal-cell; *cll*, nucleus of cell of lower lip; *cp*, centripetal marginal cell; *ct*, connective tissue; *ec*, ectoderm; *fc*, 'funnel-cell'; *fr*, group of cells from which develops the funnel = funnel-rudiment; *gc*, gutter-cell; *gr*, groove continued from canal; *lc*, leucocyte; *ll*, lower lip; *lo*, lateral lobe of lower lip; *mc*, marginal cell; *n*, nephridium; *ncc*, nucleus of central cell; *nt*, notch between central and gutter-cells; *op*, opening of canal into coelom; *p*, point at which gutter-cell meets marginal cells; *pl*, lateral edge of preseptal region; *pr*, preseptal region; *ps*, postseptal region; *r*, area without cilia continued from canal; *s*, septum; *ul*, upper lip; *v*, vacuoles in cells of upper lip.

PLATE 11.

Magnification: the scale with fig. 1 applies to all figures except fig. 7.

Fig. 1.—Longitudinal section through the middle of the nephridiostome, and beginning of the nephridial preseptal canal. Only a portion of the mass of leucocytes attached to the lower lip is shown.

Fig. 2.—Similar section through another funnel.

Fig. 3.—Longitudinal section through the funnel shown in fig. 2 passing through the lateral wall of the canal, showing the nuclei of two canal cells, and the ciliated band of the canal passing on to the gutter-cells over the edge of the opening. The arrows in this and the previous figure indicate corresponding levels.

Fig. 4.—Transverse section of the upper lip cutting through marginal cells.

Figs. 5 and 6.—Transverse sections through the upper and lower lips; fig. 5 near the edge of the lower lip, fig. 6 near its base.

Fig. 7.—Portion of a transverse section of the upper and lower lips showing the cilia of the marginal cell clinging to the edge of the lower lip.

Figs. 8 and 9.—Portions of two consecutive transverse sections of a nephridiostome showing the continuity of the ciliated band of the gutter-cells with the ciliated area of the lateral marginal cells at point, *p*. The cilia are not drawn (except in the canal) but their basal granules are indicated.

PLATE 12.

All the figures, excepting 30, 31, and 32, are taken from longitudinal sagittal sections of embryos.

Figs. 10–29, anterior on right, posterior on left. Magnification: the scale with fig. 1 applies to all figures.

Fig. 10.—Through four segments, showing young nephridial rudiments with large 'funnel-cells'.

Fig. 11.—Rather later stage, through funnel-rudiment.

Fig. 12.—Similar to fig. 11.

Figs. 13, 14, and 15.—Through funnel-rudiments at a little later stage.

Figs. 16 and 17.—Two sections through a funnel-rudiment showing first appearance of lumen, indicated by an arrow in fig. 16 and some succeeding figures.

Fig. 18. Similar to fig. 16.

Fig. 19. Later stage; upper and lower lips developing.

Figs. 20, 21, and 22.—Three successive sections through young funnel. Upper lip becoming distinct.

Figs. 23 and 24.—Two sections through slightly older stage.

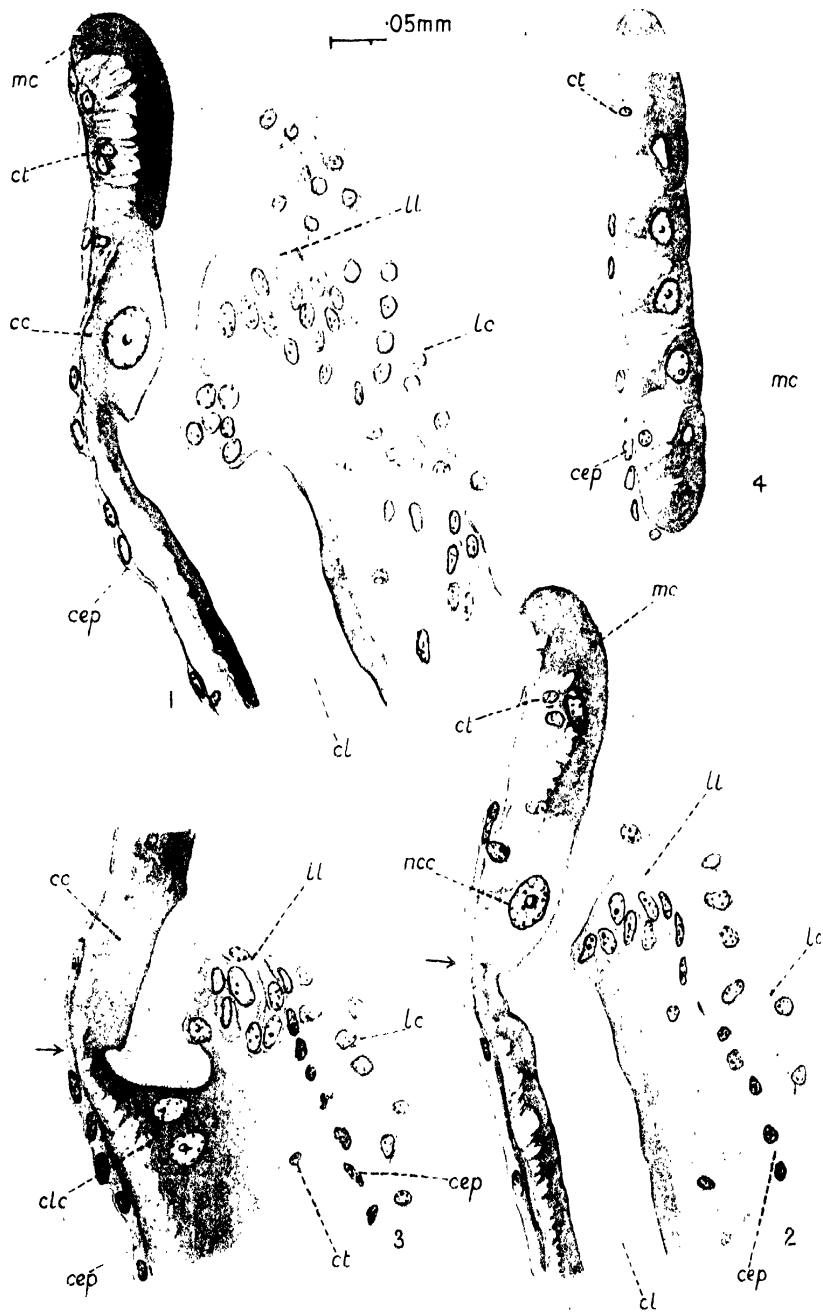
Fig. 25.—Similar stage with dividing lower lip cell.

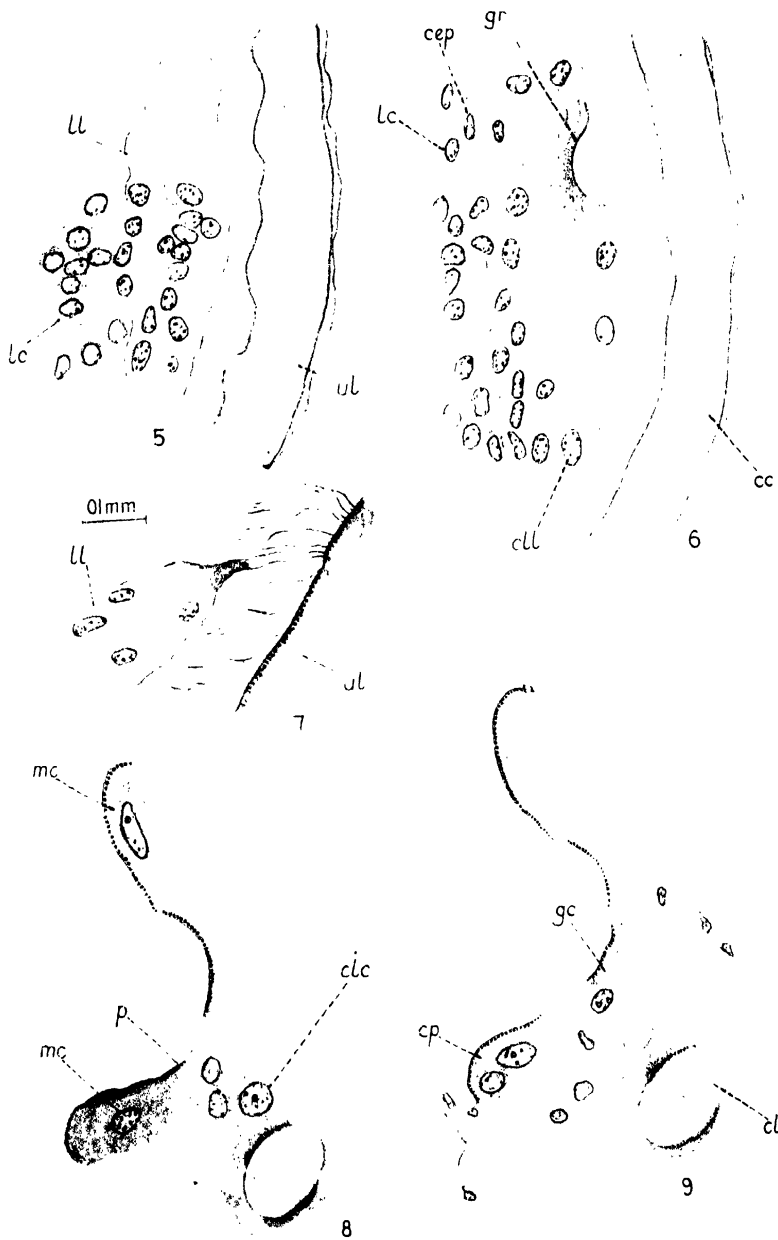
Figs. 26, 27, 28, and 29.—Four sections through older funnel with well-developed lumen, and central cell.

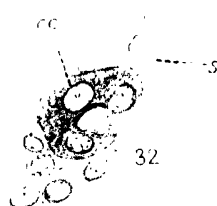
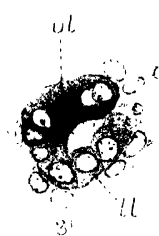
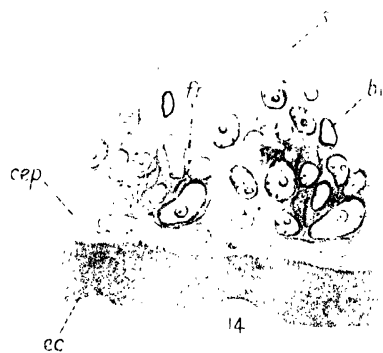
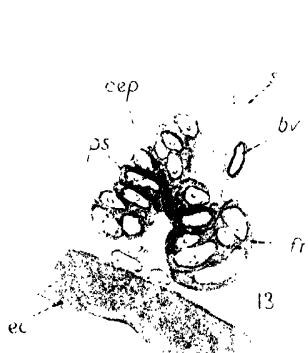
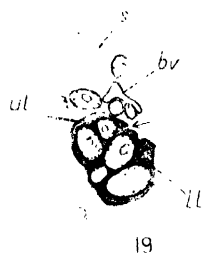
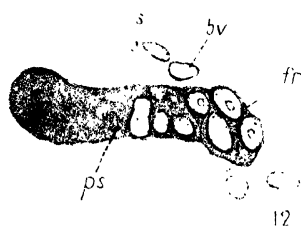
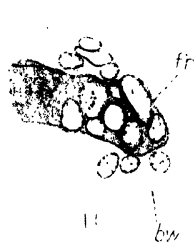
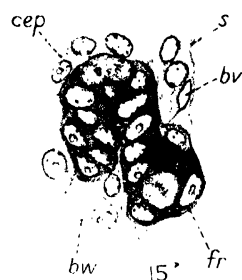
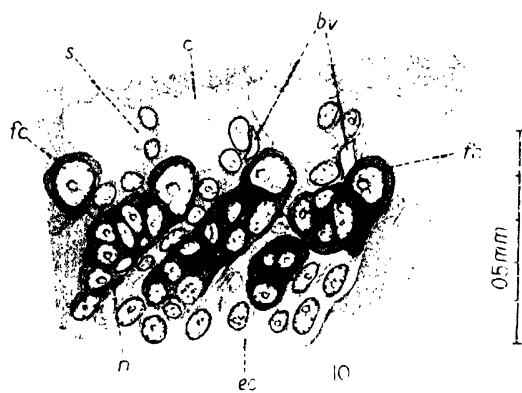
Figs. 30, 31, and 32.—Three transverse sections of a funnel, at a rather later stage.

Fig. 33.—Longitudinal section of older funnel, drawn from a 10μ thick section so that two layers of nuclei are shown.

Fig. 34.—Similar section showing many nuclei in lower lip.









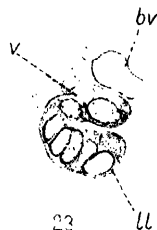
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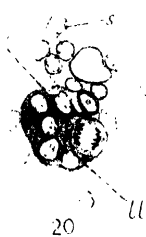
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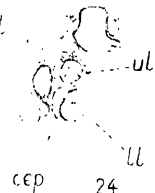
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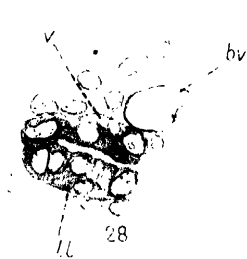
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The Innervation of the heart of the Crustacea.

I. Decapoda.

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With Plates 13-15 and 25 Text-figures.

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INTRODUCTORY.

THE innervation of the heart is one of those problems of comparative anatomy and physiology which are always being discussed with the liveliest interest. It must be stated that considering the importance of this problem our information about the plan of distribution of the nervous elements in the heart is still very scanty. With regard to the vertebrates we know that different fibres run to the heart from the central nervous system and from the sympathetic trunk; further, that many ganglion cells are present on the heart itself and abundant nerve-fibres in the muscles. But the legitimate demand for the analysis of the structural relations of all neurons which build up this very complex system gets but meagre satisfaction. The observation of preparations stained with methylene blue leads to the conclusion that the possibility of distinguishing the fibres of different provenance is very doubtful, and that the criterion for diagnosis of the several types of ganglionic cells as they have been described by some authors is more than uncertain. This opinion, to which I was led when examining many preparations of the mammals' heart, I find also expressed by Stöhr in his recent work about the involuntary nervous system.

A study of this question in the Invertebrates should yield better results, and there is no doubt that a minute knowledge of this system in the lower animals is also very desirable. It is, of course, to be borne in mind that one has to be careful when attributing general value to conclusions based on statements made in one group of animals and extending them to other groups, but it is very probable that there are analogies in the general plan of the distribution of the nervous elements, and, therefore, some light might be shed on this problem in the higher animals. Accordingly, in the larger treatises on physiology dealing with the innervation of the heart, references may be found to the lower animals, but from the examination of the bibliography on the subject it appears that our knowledge is rather unsatisfactory. There are, however, numerous accounts regarding the nerves running to the heart from the central nervous system (e.g. Carlson's) but the data concerning the

nerve elements in the heart itself are few, and even in recent works one finds now and then doubts as to the presence of ganglion cells in it. This state of things is due to the great difficulties in making preparations of the nervous system by means of all specific methods. For my own part, more than twenty years ago I tried to apply the vital staining and the silver methods in different groups of Invertebrates, such as the Tunicates, Molluscs, and Arthropods; but although it could be asserted that in all these animals the heart is provided with abundant nerve-fibres, the general staining effect was not sufficiently satisfactory.

Some years ago I repeated my attempts, and then I obtained better results with *Periplaneta* (1926) and *Potamobius* (1929). In the paper on the latter I expressed the opinion that for many reasons it would be advantageous to investigate various species of the marine Crustacea. My sojourn at the biological stations in Plymouth and Naples in the summer and autumn of 1930 made it possible to realize these plans, and I was able there to make observations on the heart in Decapods, Stomatopods, and Isopods. In the present paper I propose to give an account of my results in the Decapods.

I have much pleasure in expressing my most sincere thanks to Dr. E. J. Allen of Plymouth and Professor Dr. R. Dohrn of Naples for all the facilities which they afforded me while working in the laboratories of these two stations. I cannot refrain from expressing as well my grateful indebtedness to the Board of the Fund for the Advancement of Arts and Science in Poland, whose assistance has enabled me to pursue my researches abroad.

HISTORICAL.

On referring to the literature of the subject, we find that the chief attention, when dealing with the innervation of the heart of the Crustacea, has been paid to the nerve discovered by Lemoine in 1868, which runs alongside the anterior median blood-vessel. Other nerve-fibres approaching the heart from its sides have also been seen by several authors, e.g. Dogiel (1894), Carlson (1905), and Police (1908). The nerves of the arterial

INTRODUCTORY.

THE innervation of the heart is one of those problems of comparative anatomy and physiology which are always being discussed with the liveliest interest. It must be stated that considering the importance of this problem our information about the plan of distribution of the nervous elements in the heart is still very scanty. With regard to the vertebrates we know that different fibres run to the heart from the central nervous system and from the sympathetic trunk; further, that many ganglion cells are present on the heart itself and abundant nerve-fibres in the muscles. But the legitimate demand for the analysis of the structural relations of all neurons which build up this very complex system gets but meagre satisfaction. The observation of preparations stained with methylene blue leads to the conclusion that the possibility of distinguishing the fibres of different provenance is very doubtful, and that the criterion for diagnosis of the several types of ganglionic cells as they have been described by some authors is more than uncertain. This opinion, to which I was led when examining many preparations of the mammals' heart, I find also expressed by Stöhr in his recent work about the involuntary nervous system.

A study of this question in the Invertebrates should yield better results, and there is no doubt that a minute knowledge of this system in the lower animals is also very desirable. It is, of course, to be borne in mind that one has to be careful when attributing general value to conclusions based on statements made in one group of animals and extending them to other groups, but it is very probable that there are analogies in the general plan of the distribution of the nervous elements, and, therefore, some light might be shed on this problem in the higher animals. Accordingly, in the larger treatises on physiology dealing with the innervation of the heart, references may be found to the lower animals, but from the examination of the bibliography on the subject it appears that our knowledge is rather unsatisfactory. There are, however, numerous accounts regarding the nerves running to the heart from the central nervous system (e.g. Carlson's) but the data concerning the

nerve elements in the heart itself are few, and even in recent works one finds now and then doubts as to the presence of ganglion cells in it. This state of things is due to the great difficulties in making preparations of the nervous system by means of all specific methods. For my own part, more than twenty years ago I tried to apply the vital staining and the silver methods in different groups of Invertebrates, such as the Tunicates, Molluses, and Arthropods; but although it could be asserted that in all these animals the heart is provided with abundant nerve-fibres, the general staining effect was not sufficiently satisfactory.

Some years ago I repeated my attempts, and then I obtained better results with *Periplaneta* (1926) and *Potamobius* (1929). In the paper on the latter I expressed the opinion that for many reasons it would be advantageous to investigate various species of the marine Crustacea. My sojourn at the biological stations in Plymouth and Naples in the summer and autumn of 1930 made it possible to realize these plans, and I was able there to make observations on the heart in Decapods, Stomatopods, and Isopods. In the present paper I propose to give an account of my results in the Decapods.

I have much pleasure in expressing my most sincere thanks to Dr. E. J. Allen of Plymouth and Professor Dr. R. Dohrn of Naples for all the facilities which they afforded me while working in the laboratories of these two stations. I cannot refrain from expressing as well my grateful indebtedness to the Board of the Fund for the Advancement of Arts and Science in Poland, whose assistance has enabled me to pursue my researches abroad.

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valves are mentioned in the paper of Newmywaka (1928). Attention was drawn to the presence of nerve-cells by Berger in 1876. His discovery was confirmed by several authors, e.g. Pogoschewa (1890), J. Dogiel (1894), Nusbaum (1899), Stecka (1903), Alexandrowicz (1913), Newmywaka (1928).

In 1929 the present writer gave a description of the innervation of the heart of *Potamobius astacus* (= *Astacus fluviatilis*) distinguishing three systems of nervous elements, as will be explained later. In order to avoid needless repetition, I do not give here a detailed account of the opinions of the writers mentioned above, as their contribution will be referred to in the following chapters.

The bibliography of the foregoing investigations will also be found in the papers of Police (1908), Alexandrowicz (1913), and Newmywaka (1928).

MATERIAL AND METHODS.

The investigations recorded in the present paper were made on such different species of Decapod Crustacea as *Maia squinado*, *Cancer pagurus*, *Eriphia spinifrons*, *Carcinus maenas*, *Palinurus vulgaris*, *Homarus vulgaris*, *Scyllarus arctus*, *Munida rugosa*, *Galathea strigosa*, *Eupagurus bernhardus*, *Pagurus striatus*, *Leander serratus*. Not all of these were examined with the same exactness, for either the size of some species was not suitable for dissection of the heart or the animals were not obtainable in sufficient numbers.

The observations have been made on preparations stained with methylene blue or rongalit white.

Methylene blue Staining.—I used mostly the 'Methylenblau chem. rein, chlorzinkfrei' from Merck, Darmstadt, or 'Methylenblau zur vitalen Injektion n. Ehrlich' from Gruebler (Hollborn). As is well known, the staining of the nervous elements can be obtained in different ways, viz. by submerging the tissues in a weak solution of the dye or by injecting a more concentrated solution into the body of the living animal. By trying both these methods it was found that the nerve-cells in the ganglionic trunk of the heart are better stained when sub-

merged in a solution, while injection gives clearer preparations of the nerves of the pericardium and of the arterial valves. Being chiefly occupied with the distribution of the nerves in the heart-wall itself, I mostly applied the former method.

It was my practice to keep a standard solution of methylene blue 0.5 per cent. in distilled water, of which 15 to 20 drops were mixed with 100 c.c. of sea-water immediately before the organ was submerged. It seemed to make the preparations clearer when to the above solution a small quantity of hydrochloric acid (1 to 2 c.c. of $n/100$ HCl for 100 c.c.) was added.

Rongalit White.—The rongalit white (Rongalitweiss) was prepared according to the prescription of Unna (v. Zeitschr. f. wiss. Mikroskopie, Bd. 32, p. 302) viz.:

Methylene blue 0.5 p.c. in dist. water acidulated in

the proportion of 7 drops of 25 p.c. HCl to 100 c.c. 10 c.c.

Rongalit 0.3 gm.

Dilute and warm in a test-tube until the blue colour changes to pale-yellow; filter after cooling. As I have already indicated (Archives de Zoologie exp. et gén., t. 66, 1927) this standard solution should be kept in an open test-tube protected by a piece of paper, and may be used for about 10 days. Its staining properties are better the next day than immediately after preparation. For staining it was added to sea-water in proportion of 10 drops to 100 c.c.

I have been using rongalit white since 1924 and find that it often gives better results than methylene blue, but during my latest work I have had many failures with it, due, as I was able to demonstrate, to the quality of the drug. The last remark may be found useful by those who after the first trial will be inclined to distrust completely this method of staining. Satisfactory results were obtained by me at Naples with the rongalit obtained from Gruebler & Co.

Some measures ought to be taken for facilitating the penetration of the dye into the nervous elements. In the first place it is necessary to have the heart-wall flattened as much as possible.

To secure this the heart of the animal, previously killed with chloroform, was cut in the median line of the ventral wall. Then it was attached, the inner side upwards, to a paraffin plate

3 to 5 mm. thick with hedgehog spines; these are more advantageous than common needles, not only because of their not being acted on by different fluids but because of the possibility of shortening them as desired when observing the preparations with the microscope. The paraffin plate with the heart was put into the solution of the dye, and from time to time was taken out in order to watch the staining process under the microscope, the plate being transparent enough to permit tolerable illumination of the tissues. Usually after 15 to 30 minutes, the superficial nerve-fibres begin to stain. But even if we succeed in spreading the heart, the methylene blue does not reach all the nerve-cells, as they are included in the nerve-trunk, which is covered more or less with muscle-bundles. Therefore, in order to expose the nervous elements freely to the stain it is necessary to remove a part of the muscle-fibres. This is a very delicate operation which has to be done under the microscope with needles or fine scissors. It should be begun as soon as possible, i.e. when the main nerve-trunk becomes distinguishable; then, with the advance of the staining process, new incisions of the muscle-fibres should be made.

The objects remain attached to the paraffin plate during the whole time of staining and fixing. In order to facilitate the access of the reagents from both sides of the heart-wall a part of the paraffin may be removed, though generally I preferred not to do this.

The preparations were left in the dye for 2 to 6 hours and then, in order to obtain the more complete staining, they were exposed to the action of the air in a moist chamber. In some cases the process of staining took 20 hours in all. The point when the preparation is ready to be fixed is very difficult to determine. It is easy to say that it must be fixed when the staining is at its best, but it is not easy to know when a given preparation shows the best that could be obtained with it, especially as the nervous elements can behave very differently in the same organ. So, e.g., 10 hours may elapse between the beginning of staining of the small ganglion cells and that of the large ones, and very often the former have already lost their colour when the latter are not yet blue enough.

Injection.—The following was the solution usually employed for injection: methylene blue 0.5 per cent. or rongalit white (the standard solution) 1 vol. + sea-water 2 to 7 vol.

Of this mixture in the thorax or the abdomen of the animal 1 to 6 c.c., according to its size, was injected. After 1 to 6 hours the heart was taken out. Sometimes the animals were left alive for a longer time, viz. up to 24 hours after injection; but this method, which produced good results in *Astacus*, did not improve them in the marine Crustacea. The method of injection was serviceable for staining the system of the nerves of the arterial valves in the *Macrura* and *Anomura*, whilst in the *Brachyura* I had but little success with it.

Fixation.—It is well known that up to now there is no good method of fixing methylene blue preparations in all their beauty, and so it is understandable that every one who works with vital staining endeavours to improve the fixation of the stained nerves and tissues. Schabadasch (1930) discusses the value of different methods of fixation and, suggesting that the action of the osmotic pressure may be responsible for the conservation of the tissues, gives prescriptions for isotonic fluids with ammonium picrate as the fixing agent, declaring himself satisfied with the results. The formulae of Schabadasch are, however, not suitable for the organs of marine Crustacea, as the solution of the salts he proposes, viz. ammonium iodide or ammonium thiocyanate and ammonium picrate, cannot in practice be obtained in a concentration corresponding to the high osmotic pressure in the tissues of these animals.

In the course of more than 20 years' experience with methylene blue staining I have made many experiments in order to obtain the best fixation of my preparations, but have been obliged to confirm Dogiel's opinion that, for the most part, the simple solution of ammonium molybdate is the best. Some additions, however, as osmic acid and platinum chloride seem in some cases to be advisable and I have often made use of them previously (1909, 1913, 1927). Using mostly ammonium molybdate as fixing agent I have tried to make its solution isotonic and have found that cane-sugar may be employed for this purpose, as it does not influence the action of ammonium

molybdate in fixing methylene blue. The solution was prepared as follows:

Aqueous solution of 10 per cent. ammonium molybdate	1,000 c.c.
Cane-sugar (saccharose)	350 gm.

To this solution osmic acid and platinum chloride may also be added. The following formula was used by me for the majority of the preparations.

Solution of ammonium molybdate with cane-sugar	30 c.c.
Platinum chloride 1 per cent. in distilled water	1 c.c.
Osmic acid 2 per cent.	1 drop

To be mixed immediately before use.

The standard solution of ammonium molybdate with sugar becomes after some time more or less blue. This colour disappears after addition of osmic acid and platinum chloride.

I was able to convince myself on different organs of marine animals that the addition of sugar to the ammonium molybdate is really useful.

In the fixing solution the objects were left for from 4 to 20 hours. Then they remained in distilled water for the same time. In some cases I used for washing a solution of cane-sugar (35 gm. to 100 c.c.) and then diluted it gradually. From water the objects were brought into absolute alcohol, then into xylol and were mounted in xylol-dammar.

I also made some experiments in order to obtain a better fixation of the tissues. Of different reagents I experimented with, only formol seems not to damage the staining of the nervous tissue. Unfortunately, it gives a precipitate with ammonium molybdate solution. As this mixture still remains clear for some minutes I have profited by this property as follows: To the solution of ammonium molybdate with sugar concentrated formol was added (3 c.c. of formol to 27 c.c. of the solution) and the preparations were immediately put in. Within 10 to 20 minutes this fluid becomes turbid and has to be replaced by a freshly prepared mixture. As the preparations, being attached to the paraffin plate, swim on the surface, they do not retain the falling particles of the precipitate. The change of solution can be repeated; afterwards the objects are submerged in the

solution without formol. This proceeding is not convenient, but it is worth while trying it when the simple solution of ammonium molybdate appears quite useless. Some good preparations have been obtained by me by this method, but generally I have used the more simple one first described.

I also used fixation with ammonium picrate, but, except in some particular cases, I preferred the ammonium molybdate.

It may be added that when making and examining the preparations of the nervous system binocular observation is far superior to monocular. There are nowadays very comfortable binocular microscopes, but I preferred to use the simple Stereo-attachment¹ (Stereo-Aufsatz) of Heimstaedt made by C. Reichert, Vienna. It has some inconveniences, increasing considerably the height of the microscope;² the clearness of the pictures also does not seem to be so perfect as in binocular microscopes, but it has two great advantages. First of all, a good stereoscopic effect is obtained; preparations of the nervous system, showing the nerve-fibres at different levels, are very good for demonstrating the possibility of the spatial perception, and I find that in this respect the Stereo-attachment is superior to the modern binocular microscopes. In the second place, a special advantage of this eyepiece is that it gives an upright picture, so that manipulations with the forceps and scissors are easy. With an objective of low power (I used for this purpose objective 1 b of Reichert with a changeable magnification 3 to 4 \times), the common microscope can in many cases replace the Greenough microscope. At times even it offers some advantages, e.g. when during dissection a control with a higher power is needed this can be obtained by a single movement of the revolving nose-piece.

It may not be superfluous to call the attention of readers

¹ This apparatus is introduced into the tube of the ordinary monocular microscope and clamped fast to it. I find that in practice the screw mechanism adopted in the new pattern of Reichert is less convenient than that of the older pattern.

² In order to facilitate observation, especially in those cases when the microscope cannot be used in the bent position, I have in my laboratory a table made with an incision so that the microscope can be placed at a changeable level, 6 or 11 cm. below the level of the table.

working with similar objects, i.e. with fresh organs or with thick mounted whole preparations, to the fact that the choice of adequate objectives greatly facilitates their examination and is sometimes the only method which allows the necessary observations to be made. Of great importance when using objectives of medium or higher power is the so-called working-distance of the lens, which in ordinary histological work does not play a decisive part. As objectives of the same magnification have different constructions, I have selected, after comparing the data of several firms, such of them as have the greatest working-distance.

	<i>Objective.</i>	<i>Magnification.</i>	<i>Working-distance.</i>
E. Leitz	4	20	2.0 mm.
„	5	30	0.75 „
„	6 L.	45	0.60 „
C. Reichert	6 b	45	0.55 „
„	1/8 immers.	74	0.49 „

The first of these (Leitz 4) is seldom used in ordinary work, but for our purpose it is very advantageous, since, keeping in focus at a distance of 2 mm. from the object, it gives, in combination with an eyepiece of high power, e.g. 15 \times , a fairly good magnification. For mounted preparations an immersion objective 1/8 is very useful, as even the thicker parts can be examined with it.

DESCRIPTIVE.

I. GENERAL ARRANGEMENT OF THE NERVE SYSTEMS SUPPLYING THE HEART.

In a previous paper the writer has stated that in the heart of *Potamobius astacus* three systems of nervous elements may be distinguished:

(1) A system of neurons situated in the heart itself and therefore constituting its proper or local system.

(2) Fibres which connect this local system with the central nervous system and which are represented by the dorsal nerves—*Nervi cardiaci dorsales*.

(3) A system of fibres innervating the muscles of the peri-

cardium and those of the valves situated at the exit-points of the arteries. These are given off by nerves which will be called 'nervi segmentales cordis' and by an anterior nerve—'nervus cardiacus anterior'.

All these elements have been found also in the course of the present investigations on the marine Decapod Crustacea. Certain differences concern details only and will be referred to later. First of all an idea of the general arrangement of these nerves may be given, making use of the diagrammatic drawings representing the nerves of the heart in *Palinurus vulgaris*.

Text-fig. 1 shows the heart from its dorsal side considered as transparent so that the main parts of the local system, which lie on its inner surface, can be seen. This system consists of a stout trunk (*Tr gang*) containing ganglion cells, and of the branches arising from this trunk and distributed in the heart-muscles and the muscles of the ostia. The finer branches are not represented.

The dorsal nerves (*N dors*) enter the heart from its dorsal side and join the main trunk.

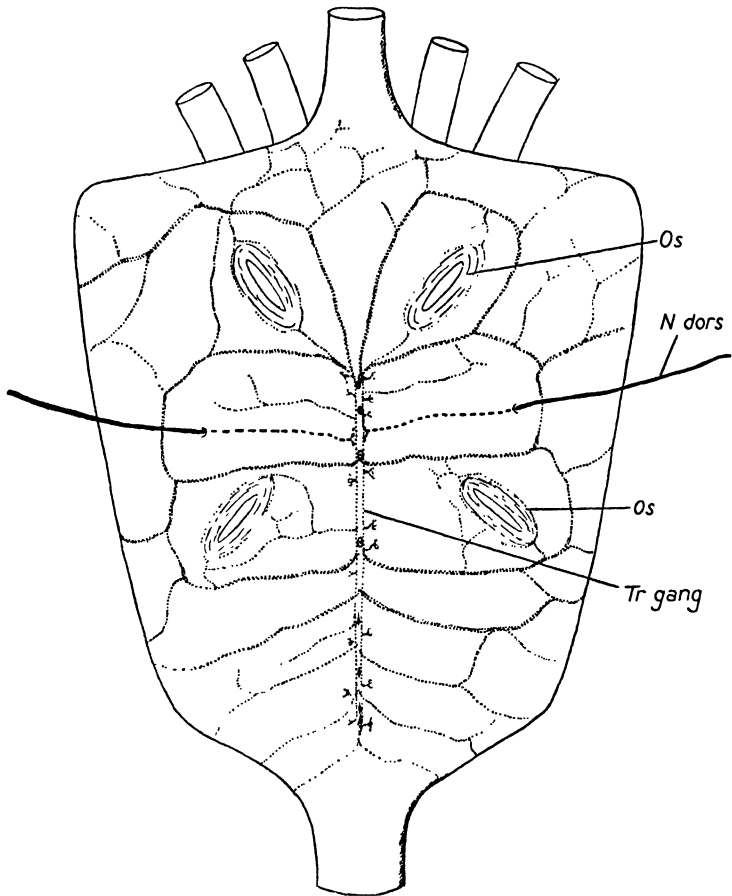
Text-fig. 2 shows the system of nerves going to the valves. The heart with the pericardium left on it is represented from its ventral side. The fibres of the local nervous system are omitted in this drawing. The nervous elements represented originate in 4 pairs of nerves coming from the sides, which I had already named segmental nerves of the heart (*Nn seg*). They unite in a bundle—fasciculus longitudinalis pericardii (*Fasc long*)—lying on the ventral surface of the pericardium. From this fasciculus—in other species they may be two in number—branches are given off whose destination is the muscles of the pericardium and the valves of five arteries, except the ophthalmic artery (aorta anterior). The valve of the latter receives its nerve-fibres from the anterior nerve of the heart—nervus cardiacus anterior (*N card ant*).

II. LOCAL SYSTEM.

1. Elements of the Local System.

The nerve-cells which with their processes build up the local system are of two sizes, large and small. Their number, as could

be ascertained for several species of marine Decapods, amounts to five large elements and four small ones. It may be emphasized



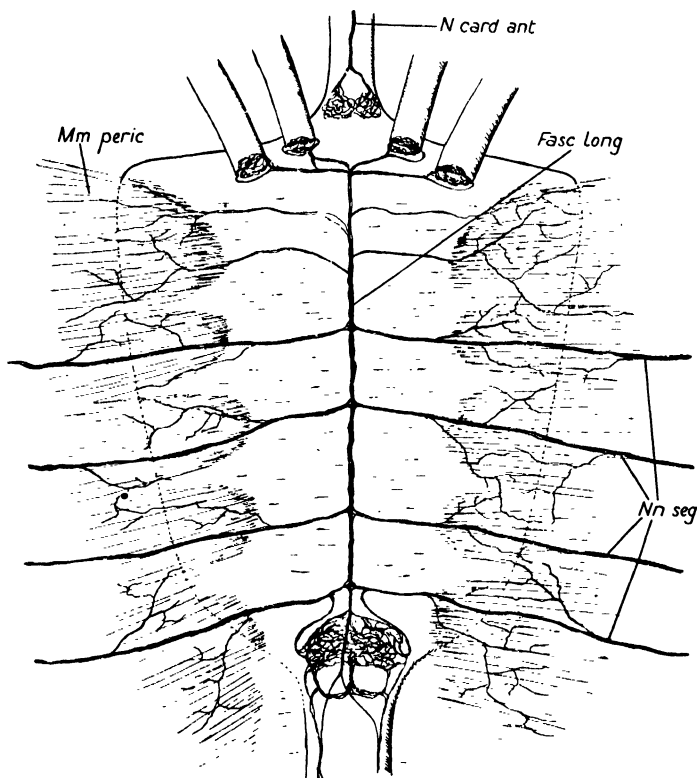
TEXT-FIG. 1.

Semi-diagrammatic representation of the nervous system in the dorsal wall of the heart of *Palinurus vulgaris*. *Tr gang*, ganglionic trunk, with its nerve-cells; *N dors*, dorsal nerve piercing the heart-wall; *Os*, ostium.

that even if that number may not be identical in all these animals it is at any rate very close to it. *Potamobius astacus*,

however, possesses, as I noted before, eight large cells and the same or nearly the same number of small ones.

The nerve-cells lie in a nervous trunk situated in the dorsal wall of the heart near to its inner surface. This trunk containing

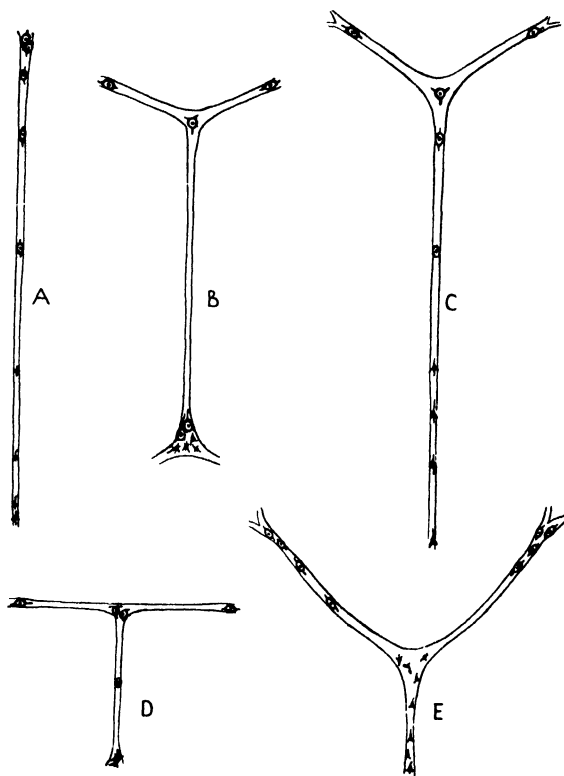


TEXT-FIG. 2.

Semi-diagrammatic representation of the nervous system of the valves and the muscles of the pericardium in *Palinurus vulgaris*. *Nn seg*, segmental nerves of the heart; *Fasc long*, Fasciculus longitudinalis pericardii; *Mm peric*, muscles of the pericardium.

the ganglion cells will, in later descriptions, be called the ganglionic trunk. It gives origin to branches passing to the different parts of the heart-wall. It is important for the staining process

that the posterior part of this trunk is not covered with muscle-bundles, while the anterior part of the trunk lies under the muscles. The latter, as has already been explained, must be removed during the staining process.



TEXT-FIG. 3.

Diagrams showing the shape of the ganglionic trunk and the situation of the nerve-cells of the local system in A, *Palinurus* and *cyllarus*; B, *Brachyura*; C, *Homarus*; D, *Galathea*, and E, *Astacus*.

There is some variation in different species with regard to the shape and relative size of the ganglionic trunk (Text-fig. 3). In the *Brachyura* (B) it is relatively shorter and bifurcates on the anterior edge. The large cells are so distributed that one of

them is situated at the point of the anterior bifurcation, two are lateral on both branches of bifurcation (fig. 8, Pl. 14), and the remaining two are placed on the posterior end of the trunk, where, too, the small cells are situated. This arrangement is by far the most frequent I met with in the Crabs, though some deviations from it have been found by examining a considerable number of the hearts of *Maia squinado* and *Cancer pagurus*. The three anterior cells may be situated either nearer to or farther from the median line, or they may lie asymmetrically; two elements on the one side and the third on the other. Further the two posterior cells or one of them may be placed in the trunk more anteriorly and in consequence at some distance from the small cells (fig. 11, Pl. 14). Variations in the number of the cells seem to be rare. In *Maia* in one preparation six large cells were found, in another five small ones. The supernumerary large cell was lying in one of the anterior branches of bifurcation of the anterior trunk. In one preparation of *Cancer* six large and five small cells were present.

With regard to *Eriphia spinifrons*, I am not sure whether this species possesses two posterior large cells or only one.

The lobster (*Homarus vulgaris*) has the median trunk relatively longer than in *Brachyura*, as it surpasses the half of the length of the heart. It bifurcates equally, forming a Y-shaped figure (Text-fig. 3, C); the disposition of the three anterior cells is similar to that of the Crabs, while the posterior cells are never found at the posterior end of the trunk, being placed nearer to the bifurcation and at some distance from one another. The small cells are situated in the posterior part of the trunk but never grouped together.

In *Pagurus striatus* the arrangement of the nervous elements in the main trunk resembles that in *Homarus*. The exact number of the small cells, however, could not be ascertained.

The trunk of *Palinurus vulgaris* is represented in Text-figs. 1 and 3, A. The microphotograph (fig. 9, Pl. 14) shows its anterior part. The trunk does not bifurcate, the large cells

being placed in one line in the anterior half of the trunk. They may be nearer to or farther from one another, the two in front apposed quite close to one another. It seems to be certain that they are always five in number. The situation of the small cells is like that in *Homarus* but their staining is not so distinct as in the latter form.

Scyllarus arctus shows the same arrangement as *Palinurus*.

Munida rugosa and *Galathea strigosa* have the trunk bifurcated and forming a T-shaped figure (Text-fig. 3, D, and fig. 10, Pl. 14). The two lateral cells are remote from the median line, in consequence of which the transverse part of the trunk is approximately of the same length as the median one.

In *Potamobius astacus* the arrangement of nervous elements differs from that represented above. The shape of the trunk is somewhat like that of *Munida* and *Galathea*. Yet the transverse part is still longer in comparison with the median, and curving forwards and outwards resembles in its outlines the antlers of a stag (Text-fig. 3, E). The large cells are placed in the transverse part and generally lie nearer the lateral edges of the trunk. The small cells are situated in the median part. There is also a remarkable difference in the number of ganglion cells, as no fewer than sixteen elements are here present. It is surprising that the arrangement and the number of the chief nervous elements in the heart of the lobster is much nearer to that of the Crabs than to that of *Astacus*. The difference in the number of the cells is really striking, if we accept the view—as I am inclined to do—that this number in the Decapods bears some relation to the number of metameres which go to make up the heart reduced in its length during phylogenetic development.

We shall give later a detailed description of the elements which are included in the ganglionic trunk, only mentioning here that it contains: (a) ganglion cells; (b) their processes; (c) the fibres of the dorsal nerves; (d) neuropile-like networks. The nervous elements of the trunk and of its main branches are bound together by a thick sheath of connective tissue.

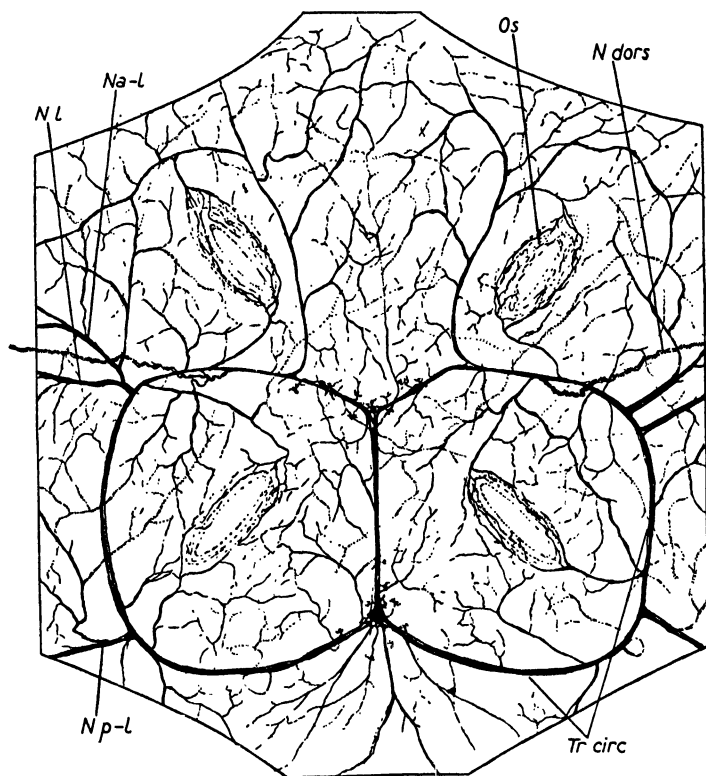
Some data may be useful regarding the size of the ganglionic trunk, but I can only give approximate values, as it is correlated with the size of the animal. In the smaller specimens of *Homarus* the trunk from its posterior edge to the bifurcation is about 1 cm., in the larger specimens up to 1.6 cm. In *Palinurus* the size is the same or even somewhat longer. In the Crabs the trunk is shorter. In large specimens of *Maia squinado*, the carapace of which was 16 cm. in breadth, it was 0.7 cm., in large *Cancer pagurus* 0.8 cm.

The general course of the nerves arising from the ganglionic trunk is indicated in the Text-fig. 4, which represents a methylene blue preparation of the heart of *Cancer pagurus* and shows the dorsal wall of the heart viewed from its inner side. The two branches originating from the anterior bifurcation go to the sides, turn backwards, and take a circular course till they reach the posterior end of the ganglionic trunk. In this way they put into communication the anterior and posterior part of the median trunk. From the latter and from the circular anastomosing branches, which consist of five stout fibres, nerves to different parts of the heart are given off. One branch—not always present—runs forwards in the median line or a little asymmetrically on one side of it. Two or three branches on each side, consisting of a small number of fibres of unequal calibre, also take their course towards the anterior part of the heart. Tracing the circular anastomosing trunks, we meet branches the direction of which is antero-lateral (*N a-l*), lateral (*N l*), and postero-lateral (*N p-l*). These branches (*a-l*, *l*, and *p-l*), which are made up of five fibres each, pass farther on the ventral wall. The nerves destined chiefly for the median part of the dorsal wall arise directly from the ganglionic and circular trunks. In the subsequent description of the cells and their processes details will be given concerning the participation of the individual neurons in these branches.

Regarding the further distribution of the nerves in the heart it may be pointed out: (1) that the branches arising from the larger trunks do not remain on one level but penetrate the heart-wall; and that the figures do not show the real course of the nerves which undergo many subdivisions at various depths,

nor do they indicate their abundance; (2) that the thick and thin branches are in different ways connected with each other, but it is difficult to give the detailed plan of these anastomoses.

It ought to be emphasized that in our diagram the nerves



TEXT-FIG. 4.

Nerves of the dorsal wall of the heart in *Cancer pagurus*. *Tr circ*, circular trunk; *N a-l*, antero-lateral nerve; *N l*, lateral nerve; *N p-l*, postero-lateral nerve; *N dors*, dorsal nerve, drawn in dotted lines; *Os*, ostium.

of the dorsal wall only are figured. It is obvious that they pursue their way on the lateral and ventral parts of the heart.

The nerves of other Crabs examined show the same plan in their arrangement, differing in some points of little importance.

In *Maia* the lateral trunks are not so regular, and the fibres both in them and in the large branches lie more closely together.

In *Homarus* and *Palinurus* the situation of the main branches does not correspond to that in *Cancer* in the following points. Firstly, more branches run laterally from the ganglionic trunk (Text-fig. 1) and, further, the large posterior branches do not arise from the end of the ganglionic trunk but forward of it. The lateral anastomoses which soon take a deeper course among the muscles are also less regular in their outlines.

In *Munida* I have found the anterior median branch more developed than in other species (fig. 10, Pl. 14).

The arrangement of the nerves in *Astacus* was figured and described in my previous paper.

It may be added that there are some other modifications in the distribution of the nerves in the various species. I will not give an account of all the details observed, as the general plan seems to be the same.

The nerves for the ostia spring from the same branches which supply the muscles adjacent to the muscles of these orifices. The nerves reach the ostium from both its angles and break up in fine fibres which stain easily in this place. The relationship of the nerves of the ostia to those of other muscles is of such a kind that I think I am justified in concluding that they form an anatomical and physiological unit (Text-fig. 5).

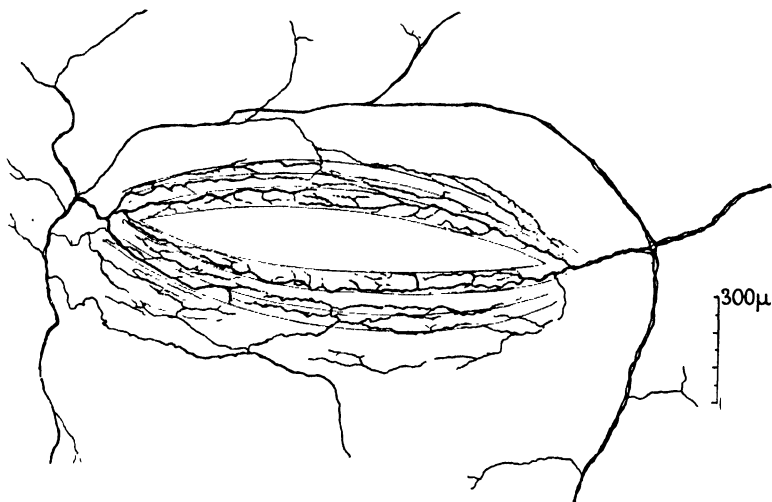
Regarding the nerve-endings in the muscles little need be said. The nerves after many subdivisions accompany the muscle-fibres as very fine fibrils. I agree with Montalenti (1926) that these fibrils end in the muscles without forming any special end-organs (Text-fig. 6).

It is to be noted that besides these terminations originating from the long branches given off by the subsequent divisions of the axons, there are others which spring from short but mostly stout branches breaking up in the muscle-bundles into a considerable number of arborescent fibres. They always lie in the neighbourhood of the ganglionic trunk and, as will be described later, may be regarded as the ramifications of the dendrites.

2. Ganglion Cells.

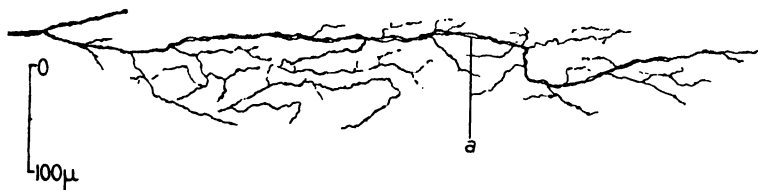
(A) Large Cells (figs. 1, 3, 4, Pl. 13; Text-figs. 7, 8, 9, 11).

The size of these elements is related to the size of the heart



TEXT-FIG. 5.

Nerves of the ostium drawn from a preparation of the heart of *Galathea strigosa*. The branches going to the ostium give nerves also to the adjacent muscles.

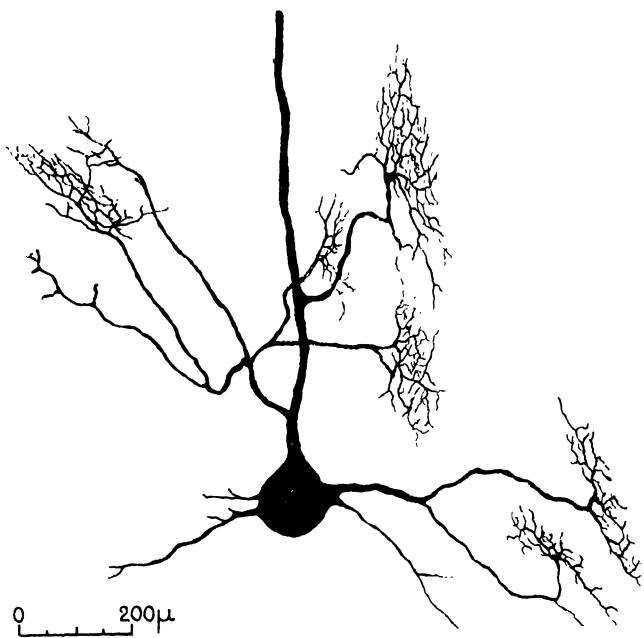


TEXT-FIG. 6.

Ramification of the terminal nerves in the muscles of the ostium of *Eriphia spinifrons*. *a*, small nerve consisting of two fibres.

and therefore to the size of the animals themselves. It is obvious that this is a consequence of the limited and constant number of the nerve-cells, each of them in the larger organs having to

supply a greater quantity of muscle-fibres. In the large specimens these cells measure up to 200μ and therefore, when stained, may be seen with the naked eye; in the smaller Crustacea such as *Eriphia*, *Munida*, *Galathea*, *Scyllarus*, they are 60 to 100μ , and thus they belong to the very large cellular elements.



TEXT-FIG. 7.

Large posterior cell of *Cancer pagurus*.

The large cells present in the methylene Blue preparations various appearances and might be classified as unipolar, bipolar, and multipolar. When several processes have stained they show considerable variations in their calibre (Text-fig. 7). There is reason to believe that all cells possess several processes some of which are seldom seen because of their refractory behaviour to the staining. Usually the anterior cells appear as unipolar or bipolar, the posterior generally as unipolar. In *Homarus*

the anterior cell situated in the bifurcation of the trunk shows as a rule three processes.

The methylene blue preparations do not reveal cytological details in these cells. The cytoplasm has a varied appearance during the staining process. At the beginning, i.e. about one hour after the organs had been placed in the methylene blue solution, numerous small granulations stained pale blue are seen in the protoplasm. They become after some time completely colourless and then the cells themselves cannot be distinguished. Only after some hours do they begin again to take little by little a deeper and uniform colour. It is probable that the former reaction occurs in the living cells, while the diffuse staining proceeds in those elements which are dying.

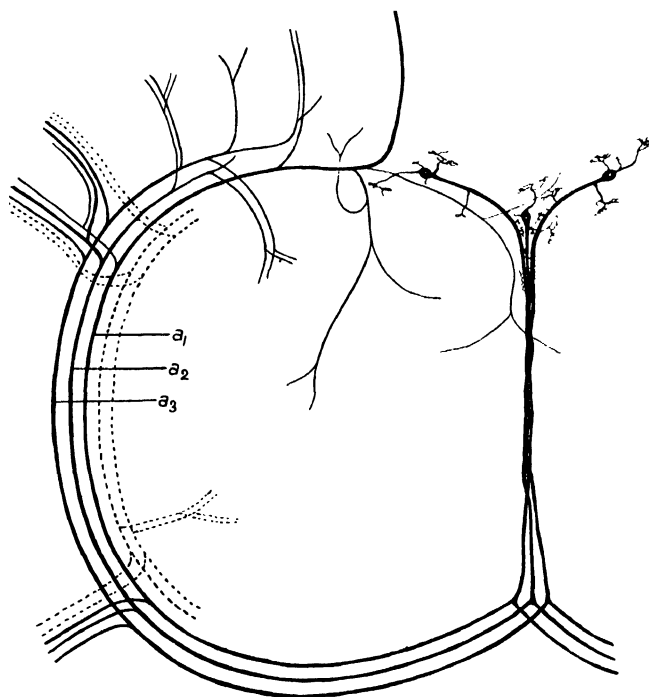
The nucleus, which is of relatively small diameter and of compact appearance, stains more deeply, but after the protoplasm has taken a deep blue colour it can no longer be distinguished in the cell.

The large cells are encased in a tissue of the same appearance as that forming the sheath of the main trunk. This tissue stains but very little with the methylene blue.

In *Cancer*, *Eriphia*, *Palinurus*, and *Homarus* numerous thin and mostly beaded fibres can be observed surrounding the cells in a kind of basketwork (fig. 3, Pl. 13, and fig. 20, Pl. 15). They are closely apposed to the cell, but whether the fibrils penetrate into it and what is the exact histology of the junction, I am unable to say. The proximal parts of the axons are often surrounded by the same fibres which enter the pericellular network (fig. 3, Pl. 13). All these elements arise from the dorsal nerves and therefore belong to the efferent system. This matter will be dealt with further in a later section.

(a) *Axons*.—The long processes of the large cells give to the heart the majority of all the nerve-elements whose distribution we have already described. As the microphotograph (fig. 16, Pl. 15) shows, the branching of the axons may present very clear images. Their whole course in the ganglionic trunk and in the main branches is very interesting, and I will give here some details observed in *Cancer pagurus*, the most favourable object for these investigations.

The axons of the three anterior cells at first run backwards down the ganglionic trunk at the posterior edge of which each of them divides into two (Text-fig. 8). In the first part of their course they give off two kinds of ramifications (Text-fig. 11):



TEXT-FIG. 8.

Diagram illustrating the course of the axons of three anterior cells in *Cancer pagurus*. In the dotted line are drawn parts of the axons of the posterior cells.

(1) short and mostly stout branches which break up in rich arborescences in the vicinity of the trunk, and which we shall call dendrites, and (2) short collaterals to the neuropiles situated in the trunk. In the microphotograph (fig. 12, Pl. 14) is represented the anterior median cell, situated here a little asymmetrically; the thick fibres (*ax, ax*) in the same figure belong to the lateral cells.

The branches of the posterior bifurcations, three on each side (a_1 , a_2 , a_3 , Text-fig. 8), take a circular course in the lateral trunks which, as was said before, run again to the anterior part of the main trunk (cf. Text-fig. 4). The postero-lateral, lateral, and antero-lateral nerves springing from the circular trunks receive fibres from each of these neurons. The fibres given off to the antero-lateral and lateral nerves are of different calibre; this fact being evidently correlated with the unequal distribution of the neurons in the heart, as may also be ascertained from their further course. The axon a_1 sends only a thin branch to the antero-lateral and lateral nerves, and taking a curved course to the median trunk emits several branches running in different directions. One of them, being the thickest of all the fibres in this part of the heart (Text-fig. 8, and fig. 12, a_1 , Pl. 14) can be traced far forward. The branch of axon a_1 , pursuing the circular course of the axon, reaches the median line and for a short distance runs backwards in it, but soon enters one of the branches arising from this trunk and passes sideways and backwards. Before doing so it sends off some fibres which cross the median line.

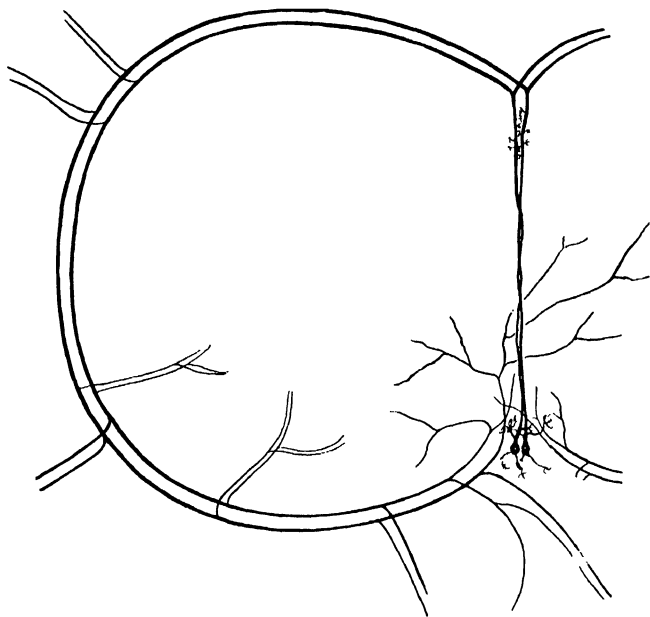
Axons a_2 and a_3 give off to the lateral and antero-lateral nerves branches of more considerable diameter. In consequence the other branches of this division running to the median trunk are thinner than that of axon a_1 . Their further course is difficult to trace with certainty among the fibres of different origin. At any rate, it can be stated that they give off dividing branches which run in various directions. Some of them accompany the branches arising from the fibre a_1 .

The branches of the posterior bifurcation, which run on the opposite side, show the same plan of division though some asymmetry on both sides may be sometimes observed.

I am unable to state to which of the anterior cells each of the fibres a_1 , a_2 , and a_3 belong, as in their course in the median trunk they could not be traced separately. It may be that axon a_1 , the territory of which seems to lie nearer to the middle line, originates in the anterior median cell.

The two posterior cells which, for the sake of clearness, are represented in a separate diagram (Text-fig. 9) send their pro-

cesses forwards in the ganglionic trunk. After giving off several shorter branches these axons bifurcate on the anterior end of the ganglionic trunk and take their course in the same circular trunks as the processes of the anterior cells, but, as is obvious, in the opposite direction. On their way they send branches to the three lateral nerves, so that these are formed of five fibres,



TEXT-FIG. 9.

Diagram illustrating the course of the axons of the two posterior cells in *Cancer pagurus*.

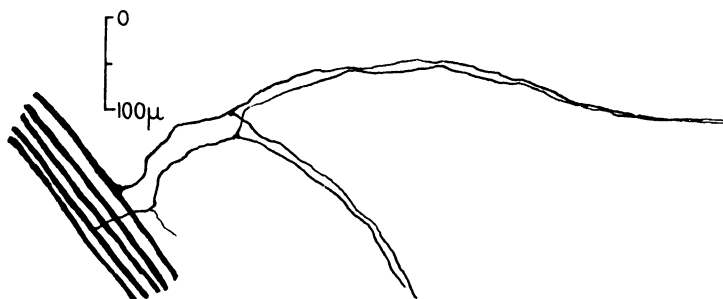
three of which belong to the system of the anterior cells and two to the posterior ones (Text-fig. 8 and microphotographs, figs. 18 and 19, Pl. 15).

Pursuing their way the axons, after giving off several branches to the posterior part of the heart, reach the ganglionic trunk and, after passing by the cells from which they have started, run forwards to end partly on the opposite side.

Our diagrams are not complete since more branches arise

from the main trunks, and I have omitted those the origin of which was uncertain.

It would without doubt be of the greatest interest to know exactly the detailed distribution in the heart-wall of all fibres springing from each of these neurons. This problem may perhaps be solved when particularly good preparations may by chance be made; but up to the present my attempts to trace further individually the longer branches have had little success as in these observations errors easily creep in. Thus I am obliged to limit my description to some points only.



TEXT-FIG. 10.

A small nerve arising from the circular trunk made up of two fibres only.

The first question was whether all nerves running to the muscles contained elements given off by all five neurons. To this question I can give a negative reply; though, as a matter of fact, five neurons are represented in the circular trunks and, as has been pointed out, several nerves arising from the trunks receive each five fibres. But in one of the subsequent divisions of these nerves branches are given off consisting of four, three, or two fibres only. Already in the smaller branches arising directly from the main trunks, various numbers of fibres had been noted. Text-fig. 10 shows a nerve given off by the circular trunks near to the postero-lateral nerves. It is formed of two fibres only belonging to the posterior cells.

The analysis of components of further divisions is difficult, the more so since anastomoses join the nerves, but the preponderance of evidence indicates that the neurons are not equally

distributed in the heart. From the examination of features represented in our diagrams the conclusion may be drawn that the axons of the anterior cells, though making a long loop and giving off many branches, predominate finally in the anterior part of the heart. The contrary is the case with the axons of the two posteriorly situated cells. On the other hand, we cannot assert that each of the neurons has to supply a definite territory isolated and independent from the others. On the contrary, it may be assumed that the area of distribution of one neuron overlaps the areas of the other neurons, since terminal branches of different origin can be observed in different parts of the heart. We see further in many preparations two fibres which belong to two neurons running in the branches of subsequent divisions parallel to each other. Even in the fine branches immediately before their breaking up in the terminal filaments the presence of two fibres sometimes can be observed (Text-fig. 6, *a*). However, there is no such regularity of double innervation such as may be seen in the muscles of the body, a fact which had already been stated by Montalenti (*loc. cit.*).

It may be mentioned here that this question is complicated by the fact that the fibres of the local system are joined by the efferent nerves. Fibres from these efferent nerves seem to run directly to the muscles. Very fine fibres which can sometimes be seen accompanying the branches of the axons of the local system might perhaps belong to these efferent nerves. On the other hand, they might be simply thin anastomosing branches of other neurons of the local system. The terminations of the fibres in the muscles have already been mentioned.

The collaterals for the neuropile are thin fibres which arise from the axons at that part of the latter which passes by the side of the neuropiles. In consequence they are of but short length. They are numerous when well stained, but this is rarely the case (Text-fig. 11; fig. 2, *col*, Pl. 13). Similar thin collaterals are given off also by the proximal parts of the dendrites.

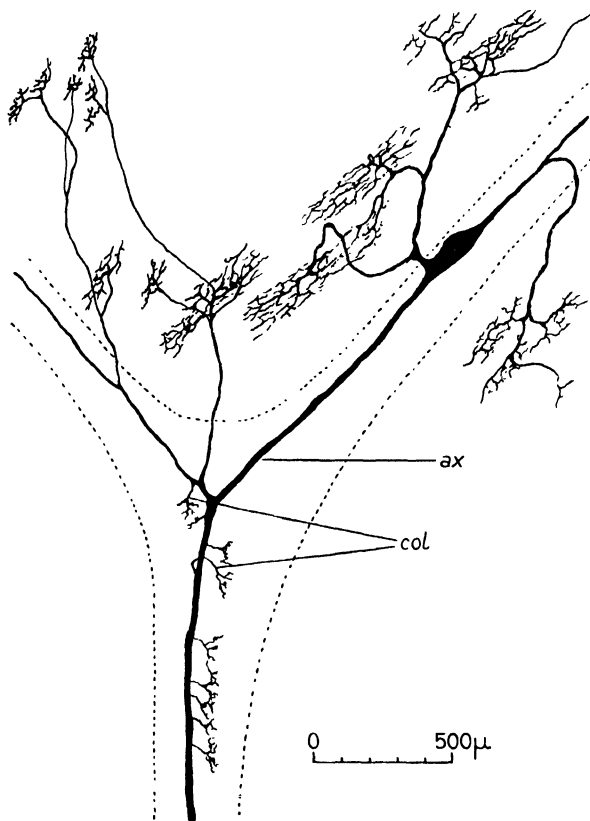
(*b*) Dendrites. Short Arborescences of the Axons.—These short branches had been observed by me for the first time in *Astacus* and description and figures are given

in my previous paper. They are like a bush or a tree with very numerous and dense short branches, so that the most typical of them can be, when well stained, at once distinguished from other ramifications of the axons (Text-figs. 7, 11; figs. 1, 3, 4, Pl. 13; figs. 14, 15, Pl. 14). They are always situated in the vicinity of the ganglionic trunk, for they arise not far from the cells. Every axon seems to send off several such branches, but it is not easy to fix their exact number especially as the thinner ones may not be of a very characteristic shape (Text-fig. 11). These arborizations penetrate among the muscular bundles and end on the muscle-fibres. The striking richness of short and continually branching nerves offers some difficulties in describing and figuring them. In their general arrangement these terminations differ from those of the long branches. Their ramifications are shorter, branch at more varying angles, and are more tightly interwoven. On the other hand, these short branches of the axons have, in their terminations, the same appearance as the short processes arising directly from the cells. Sometimes they branch at the point of exit of the axons, and in such cases might be described as projections of the axons as well as of the cells themselves (Text-fig. 11; fig. 3, Pl. 13). Therefore, I decided, not without hesitation, to give all these arborescences the general name of dendrites.

Short Processes of the Cells.—The short projections of the cells are of various size both as to their length and breadth, and, as has been said, do not stain readily. They run in various directions and, when taking their course in the large branches, are difficult to trace, so that I am not quite able to say definitely whether all these processes have similar terminations. Those, however, which go sideways are more easily observed, especially when they are of larger calibre (fig. 12, Pl. 14), and then it may be ascertained that they really end in the muscles with richly arborescent branches just as do the short branches of the axons described above.

Already during my investigations of the heart of *Astacus* it was observed that the same muscle-bundles may be connected with two short arborizations. Moreover, the presence in them of fibres, which I called accessory fibres, was assumed. After

examining different species of Crustacea I can confirm these statements. It is not easy to ascertain the relationship of such double arborescences to the respective neurons. Smaller doubts may arise when they both, as represented in the figs. 3, 4,



TEXT-FIG. 11.

Large ganglion cell of *Cancer pagurus*. *ax*, axon; *col*, collaterals.
The outlines of the ganglionic trunk are drawn in a dotted line.

Pl. 13, belong to the same neuron; but in some cases they appear as if springing from different elements (fig. 14, Pl. 14).

As to the 'accessory fibres', they are thin fibres accompanying the arborescent branches (figs. 3, 4, *ac*, Pl. 13). They belong to

the system of efferent nerves and we shall return again to these elements when dealing with the distribution of the dorsal nerves.

(B) Small Cells. (Text-figs. 12, 13, 14, 15; figs. 5, 6, Pl. 13; figs. 11, 13, Pl. 14.)

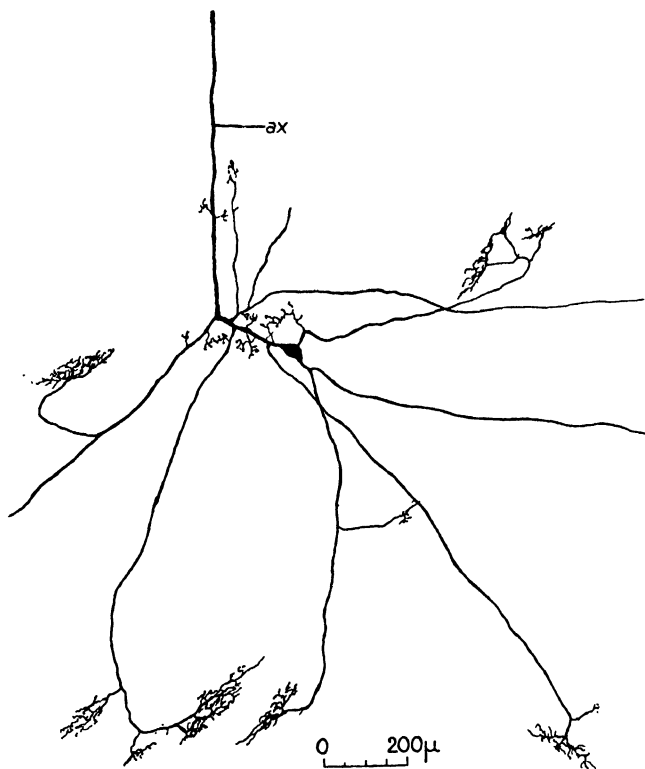
The small cells, the position of which we have already indicated, measure in the large specimens about 80μ . Therefore they differ distinctly in size from the large cells (fig. 11, Pl. 14). In smaller Crustacea, however, this difference is not always so noticeable, but, at any rate in *Astacus*, the two kinds of elements can easily be distinguished from each other. The cells are multipolar. Sometimes elements may be seen with one process only and of pyriform shape, yet this appearance is doubtless due to incomplete staining, though the cells themselves and their projections take the dye much more readily than those of the large neurons.

Two small cells of *Maia* are represented in the fig. 5, Pl. 13. In one of these cells the nucleus can be seen, but, generally, the cytoplasm, when stained well, becomes so deep a blue that the nucleus cannot be distinguished any more.

As in the large cells two kinds of processes, the long and the short, are present. In the Crabs the polar differentiation of the cells can sometimes hardly be observed, as the processes springing from them may seem to be all alike (fig. 5, Pl. 13; figs. 11, 13, Pl. 14); only from its further course can the long process—let us call it the axon—be ascertained. The short processes stain very easily in *Cancer*, more easily than in *Maia* and *Eriphia*. In the latter form the cells often appear as unipolar, and when examining them superficially one might conclude that two forms of small cells are present, one unipolar and the other multipolar. This, however, is certainly not the case.

In *Palinurus* and *Homarus* the small cells differ somewhat in shape from those in the *Brachyura*. They are more elongated, and though multipolar in fact they present often only two stained processes, one of which, the axon, runs forwards, the other springs from the opposite pole and takes its course backwards in the main trunk; the latter is nothing else than one of the short arborescences (dendrites) which is thicker than the others.

The axon of the small cells gives off short arborescent branches (fig. 17, Pl. 15) which are similar to those of the large neurons. Consequently we shall call them dendrites too. In the *Brachyura* they arise in the vicinity of the cell, while in the *Macrura* they

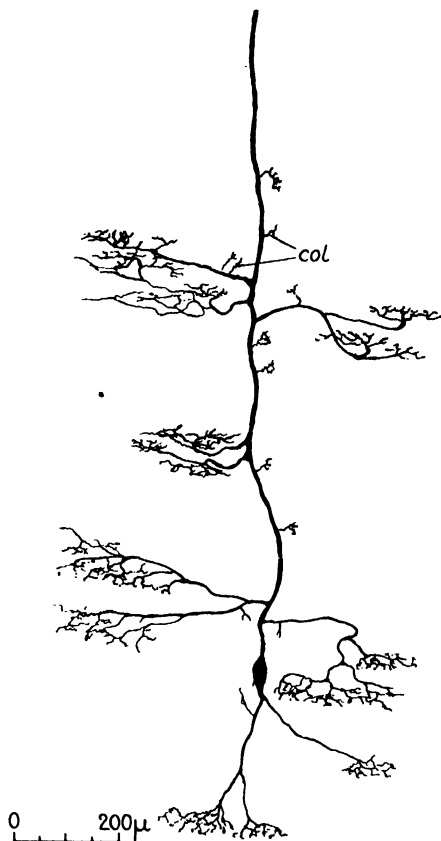


TEXT-FIG. 12.

Small cell of *Cancer pagurus*. ax, axon.

branch out at a greater distance from the cell (Text-figs. 12 and 13). Text-fig. 15 shows the small cells in *Homarus*. The situation of the cells in the trunk and the distribution of their projections should be noted. The dendrites are, as a matter of fact, more abundant than they are represented in the figure; but they rarely stain simultaneously in one and the same

preparation. Especially those running backwards in the ganglionic trunk are difficult to trace, except in the last cell, the posterior dendrites of which can often be seen distinctly, their shape being



TEXT-FIG. 13.

Small cell of *Palinurus vulgaris*. *col*, collaterals.

quite characteristic. The short thin fibres ramifying in the ganglionic trunk, which we regard as collaterals running to the neuropile, are also scattered to a greater distance if compared with their topography in the Crabs.

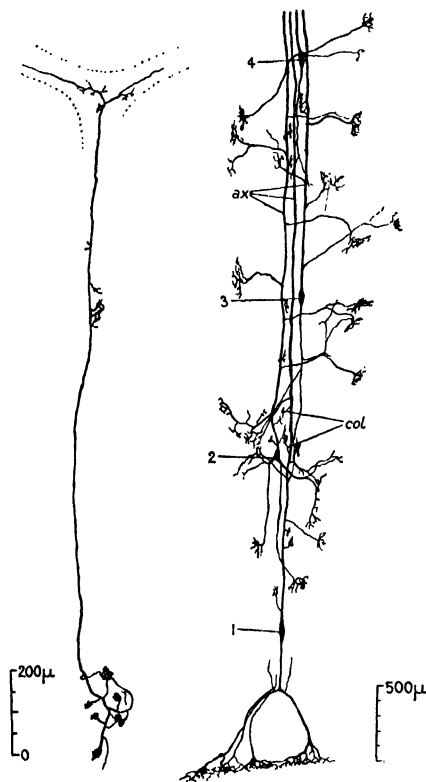
The axon pursuing its course forwards, though giving off some

branches, does not decrease in diameter and even may appear thicker at some distance from the cell than when near to it. Unfortunately, its whole course could not be traced and its final destination is uncertain. It was, however, established that it gives collaterals to the anterior neuropiles, and, on entering the anterior bifurcation of the ganglionic trunk, divides into two main branches and also gives off some arborescent branches (Text-fig. 14). The insufficiency of these observations, which is the cause of this, perhaps the greatest, gap in our present investigations, is due to the close apposition of the fibres in the ganglionic trunk, especially in those parts where they pass near the neuropiles. Any one who has had to deal with similar investigations knows quite well how difficult it is to follow one nerve-fibre for any distance without losing it among the others. In my large specimens the distance from the small cell to the anterior end of the trunk may amount to 10 mm. and more. For these investigations the smaller Crabs, as *Eriphia spinifrons*, are more appropriate.

The number of the dendrites springing from the cell body is usually from two to four; the greatest I have noticed was seven in *Maia squinado* and eight in *Astacus*. However, if we take into account the short branches originating from the proximal part of the axon, which, as we have agreed, may also be considered as dendrites, the total number of the latter will evidently be greater.

The dendrites belonging to different cells often travel parallel to each other and are accompanied by fibres of other provenance. The proximal parts of the outgrowths from even two cells present a complicated image of reciprocal relations (fig. 5, Pl. 13), in consequence of which their course is difficult to trace, especially in Crabs, in which the length of the dendrites may be considerable (Text-fig. 12). When describing these processes in *Astacus* I expressed some doubts as to their destination, but the observation of these elements in *Homarus* and *Palinurus* has cleared up the uncertainties. In these specimens the dendrites of the small cells are shorter and end in arborizations of characteristic shape (Text-figs. 13, 15). In Crabs, too, whenever the dendrites of the small cells can be followed up to their

endings, one finds them breaking up in tree-like terminations. They are found at various depths in the heart-wall but always



TEXT-FIG. 14.

TEXT-FIG. 15.

Small cell of *Eriphia spinifrons* in its whole course in the median trunk. The anterior bifurcation of the trunk is drawn as a dotted line.

Small cells of *Homarus vulgaris* (1-4). *ax*, axons; *col*, collaterals; for the sake of clearness the space between the axons is enlarged in the drawing.

entangled between muscle-fibres. I was unable to see any constant difference between the short arborescences springing from the axons and those originating in the cell itself. Moreover, they present the same features as those which we have described

before as dendrites of the large cells. There are, of course, differences in the length and calibre of the outgrowths as well as in the size of the areas occupied by the terminal filaments; but all these differences may be observed in the processes of one and the same cell and thus, from an histological point of view, all these elements appear to belong to the same class.

From the axons and the dendrites thin collaterals arise, which soon ramify in closely interwoven coils lying in the ganglionic trunk (fig. 5, Pl. 13). They are here in connexion with the endings of the efferent fibres (fig. 6, Pl. 13). The branches of the latter, too, accompany the dendrites as thin beaded fibrils. Thus the processes of the small cells have also their 'accessory fibres'.

The question now arises whether the difference between the large and small cells lies only in the size or in a totally different function. Some data of comparative anatomy seem to speak in favour of the first of these possibilities. The heart of the Decapods is derived, as is well known, from the more elongated form of the lower Arthropods and in some of these animals even bears the name of 'Dorsal pulsating vessel', which defines its shape sufficiently well. The ganglion cells of the local nervous system are in this case scattered along the heart tube. The observations concerning the topography of the cells in the heart of the Arthropods are not numerous but so far all agree as to this point. In Insects—I described the nerve-cells in the heart of *Periplaneta orientalis* in 1926, and, since then, have been able to observe the same arrangement in some other specimens—the ganglion cells are placed in two nerve-trunks accompanying the heart tube throughout its entire length. A single ganglionic trunk is present in the Isopoda, Stomatopoda, Decapoda, Xiphosura, and Scorpionidae. Very interesting is the arrangement of the nervous elements in *Squilla*. Claus (1883) has already pointed out that the nerve-cells lie at regular intervals, one cell for each segment. This writer stated it when investigating the larval form, but the same features were observed in the adult by Nusbaum (1899), and I can confirm his results.¹ From the regular arrangement of the cells in *Squilla* it appears that their number bears a constant relation to the number of segments

¹ I propose dealing with this question in a subsequent publication.

in which the heart is situated. As to the Decapods, it may be admitted that a smaller number of segments contributes to the making up of the heart, which would explain the relatively small number of nerve-cells in it.

Regarding the difference of the cell-sizes it might be suggested—this is hypothesis only—that the amount of muscle given by different metameres for the constitution of the definitive form of the heart is not the same, and in consequence the ganglion cells having to supply a greater territory become hypertrophied, while the others preserve their smaller size. With regard to the probable distribution of the neurons, we may suppose that the middle part of the dorsal wall of the heart is left for the smaller ones. According to this interpretation both large and small cells would have the same function, and the various sizes would result from the unequal amount of muscle supplied by each of the two kinds.

Two objections come to mind: firstly, some difference was noted in the staining properties of the two kinds of cells, and hence the conclusion may be drawn that they possess essentially separate functions; but another explanation of this is also admissible, viz. that these various staining effects depend on the size of the cells only; in the small elements the surface, being relatively larger, makes the penetration of the dye easier and hence the conditions of the reaction needed for the staining with methylene blue are not the same in the two cases. Secondly, the large and small cells differed in the fact that in the latter the basketwork surrounding the cells seems to be wanting. On the other hand, however, it may be recalled that in some species (*Maia*, *Potamobius*) the large cells also appear in our preparations without the fine fibres entangled around them.

The assumption that these small cells represent sensory elements must also be considered. They would differ, of course, completely from the common bipolar form of sensory cells which are well known in Crustacea, but I do not think that this fact alone renders the supposition untenable, for I have many doubts as to the completeness of our knowledge of the nervous elements in the Crustacea.¹ Therefore it is not improbable that some

¹ In this connexion it is interesting to note that in the peripheral nervous

elements may have a sensory function although their appearance does not fit into the customary scheme.

On the other hand it may be said that the behaviour of the long processes of the small cells in the heart does not support the view that they are sensory elements. Their dendrites and the axons, the latter so far as they could be traced, do not differ essentially from the processes of the larger cells; there would be also difficulty in explaining their connexions with the efferent system and even with the same branches of it if the small cells were sensory elements.

Lastly the possibility has to be considered that the small cells have in the local system some associative function but, until direct evidence has been brought forward as to their exact relationship, we must be satisfied with the statement that of the three possibilities discussed the first one, viz. that the small cells differ only in size from the large ones, is more probable than the others; yet none of them is completely excluded.

3. Historical Survey of the Problem of the Cells.

The discovery of the nerve-cells in the heart of the Crustacea is attributed to Berger who mentioned the presence of these elements in 1877. His statement was confirmed by several authors of whom J. Dogiel seems to be the first who made more precise observations by means of the gold-chloride method. From the figures illustrating his paper (1894) one may draw the conclusion that the elements represented there are certainly the nerve-cells in question, but the description given by Dogiel was inexact, for he stated that the nerve-cells in the heart of *Astacus* were grouped in two clusters ('Knoten') anterior and posterior, situated in the median line of the heart. A similar error was made by Steckla (1903) who also investigated the heart of *Astacus*. She gave a drawing in which we see large and small nerve-cells, fifteen (or sixteen?) in all, but the direction system of these animals I observed peculiar cells with processes ending in the muscles. These cells, which are undoubtedly nervous elements resembling somewhat the small cells of the heart, were found in the Decapods and the Stomatopods. In the latter, moreover, there are other unknown elements and even systems in the peripheral innervation of the abdomen. I intend to describe them elsewhere.

of the trunk including these cells as given in her description is incorrect. As to the cells stained with methylene blue in the heart of *Palaemon treillanus* by J. Nusbaum (1899) it is difficult to ascertain whether they belong to the nervous elements. The writer himself pointed out that no connexion of these cells with other nerves could be observed. It may be that in this species the staining of the nerve-trunk did not succeed and consequently the cells appear as isolated. In 1913 I described ganglion cells in *Palinurus vulgaris* and *Carcinus maenas* which could be made out by means of the methylene blue method. Their nervous character and the presence of the connexions with the nerve-trunk were beyond doubt, but the distribution of the local neurons in the heart and their relation to the efferent system were not traced out. Newmywaka in 1928 investigated the heart of *Astacus*, using also the methylene blue method. The greatest number of cells stained by him in one preparation was sixteen, but Newmywaka expresses the opinion that these cells are probably much more numerous. This writer figured the nerve-cells as unipolar, bipolar, and multipolar. As to their function he says: 'Es ist sehr möglich, dass diese Zellen einen diffusen rezeptorischen Apparat vorstellen, welcher den rezeptorischen Endigungen im Herzen der Wirbeltiere analog ist.' The results of my investigations on the same subject published in 1929 have been referred to in the foregoing description.

III. NERVI CARDIACI DORSALES.

The dorsal nerves (regulator nerves of the heart) consist of fibres by means of which the heart communicates with the central nervous system. They certainly contain the efferent fibres conveying the impulses from the infra-oesophageal ganglion to the heart. Whether there are also others which, as afferent fibres, run in the opposite direction I have no positive knowledge. The term 'dorsal nerves' was proposed by the writer when describing this system in *Astacus*, as these nerves coming from the sides pass to the dorsal wall of the heart and penetrate into it. The addition 'regulator nerves' seems to be advisable owing to the fact that in the meantime in another

group of Crustacea, namely, in Isopods, nerves had been found, which are evidently homologous with the 'dorsal nerves' of the Decapods but differ in their topography.

1. Historical.

The nerves which reach the heart from the sides and influence its rhythm were investigated by J. Dogiel in 1894, who noted that before him Eckhard in 1367 had made some experiments on a nerve having an inhibitory action on the heart. J. Dogiel, by his own physiological experiments, was led to the conclusion that two kinds of fibres, inhibitory and accelerator, run to the heart of *Astacus*, yet from the drawing he gives it is not clear whether the nerve he found in the microscopical preparation really represents the fibres in question. Much more precise are the observations of Carlson (1905), who described the course of two nerves springing from the large thoracic ganglion and illustrated the results of his investigations by a diagram which is well known from several reproductions in works dealing with the comparative physiology of the heart.

G. Police in 1908, unaware of Carlson's paper, described in *Maia* three pairs of nerves approaching the heart from the sides. One pair of them, figured by this writer in *Maia* and *Scyllarus* and called by him *nervi cardiaci*, seems to correspond to our *nervi dorsales*, although their relation to the heart and to the other nerves is represented in a different manner from that which we shall describe later. Police has also endeavoured to find out the origin of these nerves and says that they spring 'con tutta probabilità' from the infra-oesophageal ganglion, being independent of the 'visceral nervous system'.

2. General arrangement of the dorsal nerves.

The dorsal nerves can be well observed when, after the injection of methylene blue or rongalit white—in the *Macrura* the staining succeeds more easily—a part of the dorsal carapace is taken off and the heart exposed. When the staining is favourable a pair of nerves is to be seen branching from the nerves running on the thoracic muscles. They cross the lateral border of the heart at about the middle of its length, pass on the

dorsal wall and penetrate it approximately midway between the lateral border and the median line.

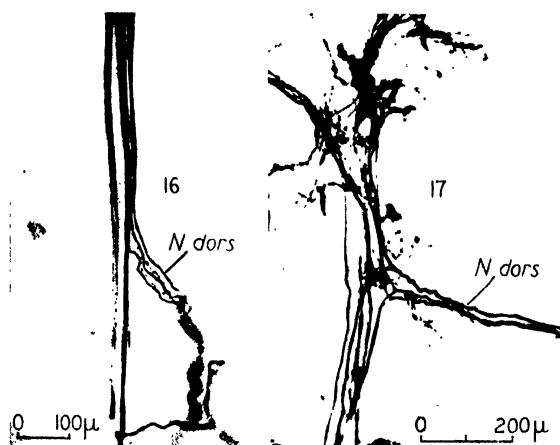
In some rare cases a branching of these nerves was observed at some distance from the heart and in consequence two branches reached the ganglionic trunk, but I could not as a rule find two nerves on each side.

It is a very difficult task to find out the origin of the dorsal nerves by anatomical methods. They branch, as was said, from the nerves lying on the muscles of the epimeral plates; by following the latter nerves we find that they spring from the infra-oesophageal ganglionic mass. But precisely these nerves are interconnected by numerous anastomoses, and, therefore, I was not able to trace with certainty the course of the heart nerves in them. According to Carlson the inhibitory nerves for the heart take their origin 'near the roots of nerves to the third maxilliped and the accelerator near the roots of the first ambulatory nerves'. Thus, the heart nerves have different roots, but whether they run independently up to the heart seems to me more than uncertain. In all species I could see one pair of nerves taking part in the innervation of the heart itself and therefore, if it is not a peculiar case in that another nerve remains always resistant to the staining with methylene blue, I am inclined to conclude that the fibres originating from different roots join in one bundle, i.e. our dorsal nerve. The structure of the nerve itself, which is composed of two kinds of fibres of different calibre, is not incompatible with this interpretation.

Mention may be made here of particular swellings on the dorsal nerves just before they enter the heart. I observed them in *Astacus* and called them 'apparatus nervi dorsalis'.

After piercing the heart-wall the dorsal nerves join the local nervous system (Text-figs. 2, 4). It is not difficult to trace their course through the wall (fig. 7, Pl. 13) and after some experience to distinguish them from the branches of the local system as they differ somewhat in their appearance (*N dors*, Text-figs. 16, 17). This difference may be yet more pronounced owing to the unequal staining reaction of the two systems in consequence of which the fibres of the dorsal nerves generally take up the dye sooner (especially in *Eriphia* and *Palinurus*) or, when

stained, differ sometimes in colour from the fibres of the local system. These properties enable us to follow them in favourable cases through their whole course in the ganglionic trunk. However, this relative facility in examining the dorsal nerves does not apply to all their elements but merely to one kind, viz. the fibres which, as will be seen, communicate in the ganglionic trunk with the cells and their projections. For the sake of



TEXT-FIG. 16.

TEXT-FIG. 17.

Point of junction of the dorsal nerve (*N dors*) with the trunk of the local system in *Cancer pagurus*. Microphotograph. The same as Fig. 16 in *Palinurus vulgaris*. Three fibres of the dorsal nerve (*N dors*) join the trunk and divide in a T-shaped figure.

facilitating the description I will call them 'System I', which is made up of the thicker fibres. The thinner elements we shall call 'System II'.

3. System I of the dorsal nerves.

The fibres of System I, after entering the ganglionic trunk, run throughout its whole length. In the species in which the trunk bifurcates at its anterior end they pass, following the branches of bifurcation, to the median line and, after reaching it, turn directly backwards. In *Palinurus* and *Scyllarus*

their course is somewhat different: as the microphotograph (Text-fig. 17) shows, each fibre on arriving at the ganglionic trunk divides into two, giving rise to a Y- or T-shaped figure, and sends one branch of this division forwards and the other backwards.

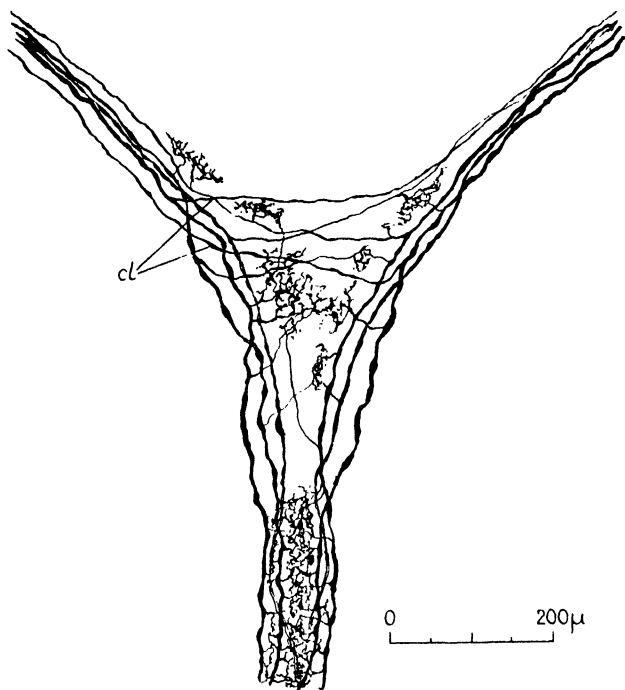
The fibres which make up System I are not numerous. In *Palinurus*, where the relations between the ganglionic trunk and the dorsal nerves are easy to examine because of their position, some preparations show three fibres on each side at the point of junction of the dorsal nerves with the ganglionic trunk (Text-fig. 17). This has been the largest number seen; usually only one or two of them have stained.

The characteristic feature of the fibres of System I is the abundance of thin and richly ramifying branches meeting in the ganglionic trunk and in its neighbourhood and giving off neuropile-like networks of fibrils. The latter establish connexions between the fibres of System I with each other, and between these fibres and both the large and the small neurons of the local system.

The connexions between the fibres of System I show a striking abundance and density of arborescences. The neuropiles which we have mentioned when describing the ganglionic trunk appear as if they were made up chiefly of these branches. The networks are situated in the Crabs mostly in the anterior part of the trunk, forming here one or, more rarely, two oblong masses and some smaller ones, the latter in the bifurcation of the trunk or in its main branches. Fig. 2, Pl. 13, represents a part of the larger neuropile in *Maia* and the numerous short collaterals arising from the fibre belonging to the System I. In Text-fig. 18 we see the networks and the shorter and longer branches reaching them. Observation of fixed and fresh preparations—the latter are more convenient for this purpose as the fixation of the neuropile is difficult and seldom gives clear images—teaches us that branches are sent to these neuropiles by different fibres from the same side, as well as from the opposite one.

In the posterior part of the ganglionic trunk of the *Brachyura* the same fibres of the dorsal nerves give off again networks of fibrils smaller than those in front (fig. 6, Pl. 13).

All these neuropiles may have various appearances in fixed preparations. Sometimes they present small irregular plates with uneven outlines and very fine granules; in other preparations the granulations are larger. As a matter of fact, these



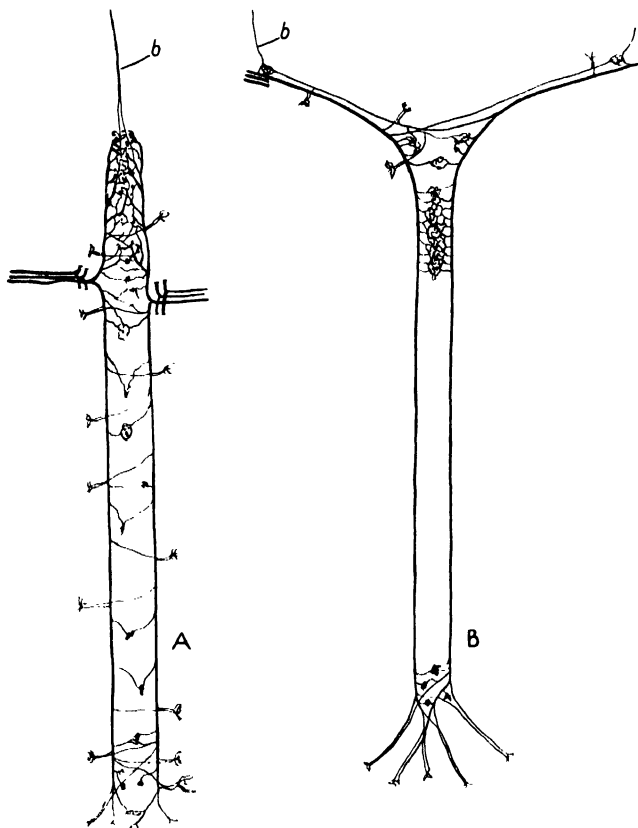
TEXT-FIG. 18.

Fibres of System I of the dorsal nerves in the anterior bifurcation of the ganglionic trunk. The figure is drawn from two preparations of *Cancer pagurus*; the same arrangement is found in other Crabs. Some of these fibres can also be seen in the microphotograph, fig. 12, Pl. 14. *cl*, fibres crossing the median line and entering the contralateral branch of the trunk.

neuropiles consist probably of very dense networks of fibrils, which by the action of reagents are deformed in different ways.

In the *Macrura* the branching fibres of System I of the dorsal nerves do not give such convoluted masses and the networks, though very numerous, are arranged more loosely along the

ganglionic trunk, yet they are denser in the anterior part of it. The diagrammatic figures (Text-fig. 19), in which only one fibre



TEXT-FIG. 19.

Diagram showing the course of the fibres of System I of the dorsal nerves in *Palinurus* (A), and in *Cancer* (B). Only one fibre on each side is represented. *bb*, fibres of unknown destination arising from fibres of System I.

on each side is represented, may illustrate the course of the System I in *Palinurus* (A) and *Brachyura* (B).

The fibres of System I travelling in the ganglionic trunk send out branches which are not confined to this trunk only. Some

run sideways and soon ramify between the muscle-bundles. It is easier to observe them in the *Macrura*. As shown in the micro-photograph (fig. 21, Pl. 15) these branches may consist of several fibres which come into close relation with each other. In *Eriphia* I observed these fibres ending in small coils.

It has been mentioned that in some preparations the dorsal nerves had already stained before all the others had taken the dye. In such a case the reciprocal connexions of all the fibres belonging to System I prevail to such a degree that one might assume that the function of all neuropiles is the mutual exchange of the impulses among the fibres of the dorsal nerves only. This is certainly not the case, all these structures being at the same time the fields of conjunction between System I of the dorsal nerves on the one hand and the neurons of the local system on the other.

There are various parts of the large neurons which are in close relation to System I, viz. (a) the cell bodies; (b) the dendrites; and (c) the collaterals to the neuropiles.

(a) In one of the foregoing sections when dealing with the histology of the nerve-cells, we described the pericellular networks which are made up of varicose fibrils. In *Cancer*, *Eriphia*, *Homarus*, and *Palinurus* these networks stain distinctly (fig. 20, Pl. 15) and I could convince myself that they belong to what we call System I, from which many fibres ramify round one cell. The most probable destination of a part of the branches of the dorsal nerves which cross the median line, as they are represented in the Text-figs. 18 and 19, B, is participation in the network surrounding the contralateral cells.

(b) The branches of the dorsal nerves accompanying the dendrites have also been mentioned before. Already when dealing with the heart of *Astacus* it seemed probable to me that the so-called accessory fibres (figs. 3, 4, *ac*, Pl. 13) of the short arborescences belong to the dorsal nerves. This assumption seems to be correct, for the connexions of the dendrites with the branches of the dorsal nerves were often observed; but, unfortunately, their relations cannot be discerned in detail. When the ramifications of the dendrites have stained well and are then very numerous, there is no possibility of tracing the finer fibrils

separately. I am, therefore, unable to say whether all the arborizations of the dendrites are accompanied by fibres of the dorsal nerves. The observations are rendered much more difficult by the fact that the dendrites rarely stain and mostly not at the same time in both systems. Many branches of the dorsal nerves which run sideways from the ganglionic trunk may be nothing else than these accessory fibres of the dendrites, the latter not having stained in this place. If such be the case we can assume that one dendrite may be in relation to the branches of different fibres of System I.

The thin collaterals given off by the proximal parts of the short arborescences to the neuropiles have already been mentioned.

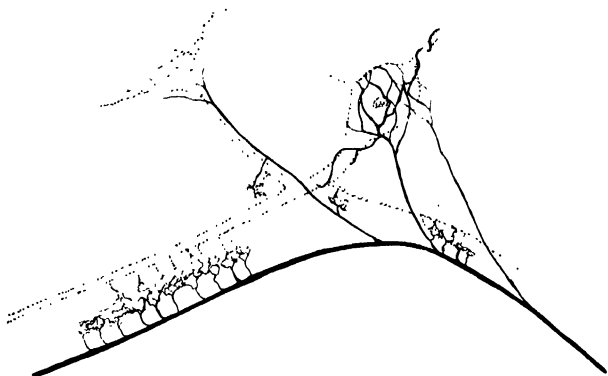
It remains doubtful whether the 'accessory fibres' only accompany the dendrites or continue farther to other nerves or, perhaps, to the muscles. Thus, for instance, in the anterior part of the trunk thin fibres may be noticed which leave the trunk and go with the nerves of the local system (Text-figs. 19 A, 19 B, b). It is not improbable that they accompany one of the longer dendrites of the anterior cells. Alternatively, they may represent fibres of System I with some special unknown signification.

(c) The further fields of conjunction between the dorsal nerves and the neurons of the local systems are the neuropiles, and it seems most probable that this is the essential role of these networks. The collaterals of the axons are short and thin, and in their shape do not differ much from those of the dorsal nerves (fig. 2, Pl. 13), but they do not stain readily and even when stained remain mostly pale blue. In view of this fact we may conclude that the predominance of the fibres of the dorsal nerves in the neuropiles is only apparent and due to their staining properties.

The connexions with the small cells are, properly speaking, the same as with the large ones, except the network surrounding the cells, though fibres approaching the cells and lying on them can be observed. As to their dendrites they are accompanied by the 'accessory fibres' branching from the dorsal nerves. Junctions by means of networks to which both elements send thin fibres are also present. Fig. 6, P. 13, represents a

small cell of *Maia squinado* showing the connexions by means of small neuropiles in the ganglionic trunk.

According to this description, when we summarize all that has been said about System I of the dorsal nerves we come to the conclusion that at least the majority of its fibres end in synaptic junctions with the neurons of the local system, as illustrated by the diagrams, Text-figs. 20, 21. The neuropiles seem to be the largest field of these conjunctions. The question arises whether all cells send collaterals to the same part of the neuropile, when it



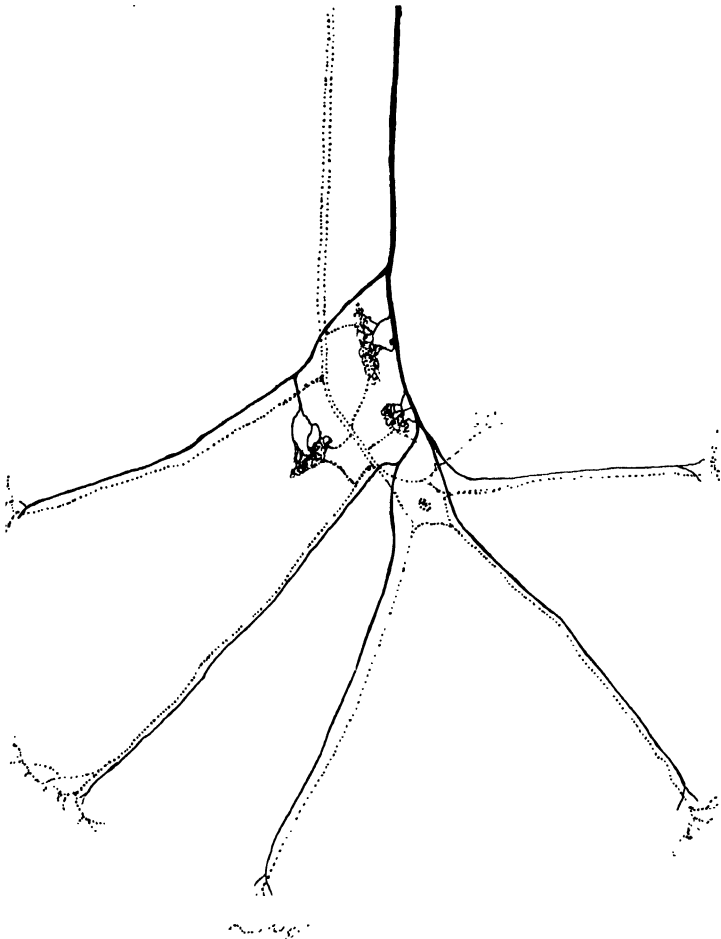
TEXT-FIG. 20.

Diagram showing the relation of a fibre of the dorsal nerve to the large cell.

forms such a convoluted mass as seen in *Brachyura*. I am inclined to answer this question in the affirmative, although this statement, so far as it concerns the small cells and the two larger ones which are situated in the posterior end of the trunk, cannot be made without some reservation, owing to the distance of these elements from the anterior part of the trunk and the possibility of errors in tracing the fibres. However, in some preparations, I was able to observe the collaterals lying far from the cell-body (Text-fig. 14).

4. System II of the dorsal nerves.

The fibres of the dorsal nerves which do not belong to System I are of smaller but not equal calibre. The term System II



TEXT-FIG. 21.

Diagram showing the relation of a fibre of the dorsal nerve to the small cell.

employed in this description is used only in order to distinguish these fibres from the former system and does not imply that they belong to one and the same anatomical and physiological unit, as I am unable to give a more detailed account of their distribution in the heart. The nerves in question do not stain

satisfactorily, and even when stained cannot be traced with certainty among the other fibres. Some features only can be mentioned here.

In *Astacus* I found fibres of the dorsal nerves which, arriving at the trunk of the local system, take a separate course running in the opposite direction from other fibres of the dorsal nerve towards that part of the heart where no cells are present and ramifying between the muscles.

Similar bifurcation of the dorsal nerves could be observed in *Scyllarus* and *Munida*, and also, in some rare cases, in *Palinurus* and *Cancer*. This might be regarded as an accidental aberration of the usual course of the fibres. On the other hand, comparing the dorsal nerves before they join the trunk of the local system and after, it must be asserted that some of the fibres are lost somewhere, and, therefore, the cases in which they are found to be taking a separate course might indicate the real destination of these elements. Part of the thinner fibres accompany the fibres of System I in their course towards the ganglionic trunk, but I could not trace them farther. Possibly, those fine fibres which are sometimes found along the long branches of the local system are of this origin. If I am right in this conjecture, the System II, or a part of it, may be considered as consisting of fibres of efferent nerves which go to the heart muscles without giving synapses with the nerve-cells in the ganglionic trunk.

In Fig. 7, Pl. 13, the dorsal nerve of *Palinurus* is represented at the place where it pierces the heart-wall (indicated by the line *a-a*). The fibre S II ramifying on the inner surface of the heart-wall belongs to System II.

IV. APPARATUS NERVI DORSALIS AND THE NERVES OF THE PERICARDIAL CAVITY.

In *Astacus* I had observed on the dorsal nerves peculiar short branches ending in irregular plates or cell-like bodies. I have called them 'apparatus nervi dorsalis' and suggested that the apparatus may be concerned with the appreciation of the pressure in the pericardial cavity. In *Eriphia* and *Scyllarus* cell-like bodies, and in different species thin

ramifying fibrils surrounding the dorsal nerves were seen in this situation. But so far I am unable to give an exact description of these elements and of their relation to the dorsal nerves. Possibly they form a part of peculiar terminations of the nerves entering the pericardial cavity, for in addition to the pair of dorsal nerves (regulator nerves), several other nerve bundles can be seen—especially numerous in *Palinurus* and *Scyllarus*—which spring from the thoracic nerves, and running to the dorsal surface of the heart, break up in a fine arborescence of thin fibrils which assume a beaded form. This fact suggests that the heart itself is supplied with many nerves, but microscopic examination convinces us that only one nerve-bundle on each side, viz. the dorsal nerve, penetrates the heart-wall, while the others remain in the pericardial cavity and terminate on the ligaments and in the connective tissue on the dorsal surface of the heart. Methylene blue does not give clear images of these endings, since the thin fibrils which originate from the thicker fibres actually present a mass of blue points like the ‘punctate substance’ of the neuropiles (fig. 7, Pl. 13).

Similar structures can be seen on the epimeral plates and I had previously observed them in *Potamobius*. In Stomatopods they are present in the abdomen and are in connexion with a peculiar nerve which I think is homologous with Newport’s nerve in Insects. I think also that all these systems of peripheral neuropile-like structures must be regarded as sensory elements which may have some relation to respiration and to the circulation of the blood in the body cavities. The matter needs further investigation.

V. NERVES OF THE ARTERIAL VALVES AND OF THE PERICARDIAL MUSCLES.

The valves situated at the entrances to the arteries arising from the heart are innervated, except the valve of the anterior median vessel, by branches of the nerves which I called *nervi segmentales cordis*. The same nerves give off branches to the muscle-fibres of the pericardial plate. The valve of the median artery is supplied with a nerve which runs alongside that vessel—*nervus cardiacus anterior*.

1. *Nervi segmentales cordis.*

I adopted this term describing the innervation of the heart in Insects in order to point out their origin from different segments. The system found in Crustacea may be considered homologous with that of the Insects. In the Decapods, according to the shape and position of the heart, its segmental nerves are confined to the thoracic region only, whilst the segmental nerves in the abdomen run to the abdominal dorsal vessel and from that point might be called *Nervi segmentales aortae abdominalis*.

As regards the number of the segmental nerves of the heart, I have found in *Astacus* four of them on each side, and I think that the same number is possessed by the marine Crustacea also, but, since the latter specimens had been used principally for the investigation of the local system, my observations on segmental nerves have been more numerous in *Astacus*.

The methods employed in making the preparations demonstrating this system were somewhat different from those indicated above for staining elements of the local system. The segmental nerves take the dye much more readily when it is administered by means of an injection, and therefore this method of staining them was chiefly applied. Some hours after the injection the carapace must be removed from the thorax, and then the liver, the genital organs, the stomach, and the intestine must be taken out. The pericardium must be left intact and remains attached to the thoracic muscles. After longitudinal section of the ventral wall of the thorax, the underside of the heart and the segmental nerves on it can be seen. The whole preparation can then be fixed in ammonium picrate or ammonium molybdate. It is possible after washing to detach the muscles from the chitinous parts and mount the whole, i.e. the heart with the pericardium and the adjacent muscles. Then, the segmental nerves in connexion with the thoracic nerves from which they branch may be observed.

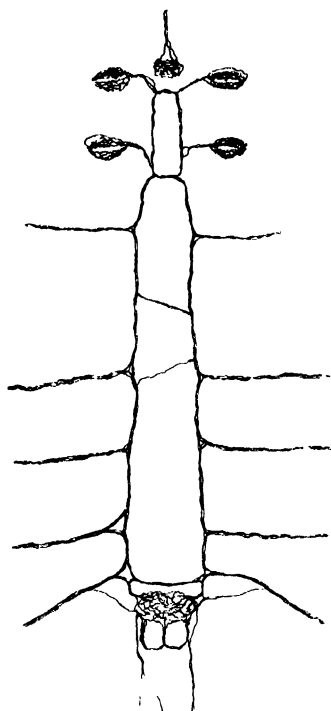
In the *Brachyura* I could not obtain similar preparations, this failure being due at first to the much less satisfactory staining of these nerves in the Crabs. On the other hand, tracing the

nerves is very difficult owing to the form of the chitinous parts of the thorax. The system of segmental nerves is of course also present in the *Brachyura*, and, so far as I could determine, is arranged similarly to that of the *Macrura*. In *Astacus* I found that the segmental nerves of the heart travel in the thoracic nerves II to V (denomination according to Keim, 1915). The point where they branch from these nerves may be easily observed, whereas their proximal course in the thoracic nerves could not be traced farther. Passing on to the pericardium they give off fibres to the muscles of the latter and running farther towards the middle line join in a longitudinal nerve bundle (Text-fig. 2). In a foregoing paper I called this bundle *fasciculus medianus pericardii*—as in *Astacus* and *Palinurus* it was found lying in the median line. However, in *Homarus*, *Scyllarus*, and in the *Brachyura*, there are two bundles directed more or less longitudinally (Text-figs. 22, 23), and, therefore, the name *fasciculus* (i) *longitudinalis* fits better. In this bundle (or bundles) fibres meet from the segmental nerves and, taking various courses, are distributed among the branches running (1) to the pericardial muscles which, therefore, receive fibres directly from both the segmental nerves and from the longitudinal bundles, and (2) to the valves of 5 arteries, viz. the *arteriae antennales*, *arteriae hepaticae*, and *aorta posterior*. The microphotographs (figs. 22, 23, Pl. 15) show the longitudinal bundle and the nerves going to the pericardial muscles in *Palinurus*.

The nerves on arriving at the valves break up into a tuft of fibres which, ramifying again, are closely interlaced between the muscles. The arborizations of these nerves mark well the outlines of the valves and, in the two pairs of the anterior arteries, the longitudinal aperture between two parts of the valve may be seen (Text-figs. 22, 23). The nerves of the posterior valve have a much more complicated course, which I have described in *Astacus*. This is due to the peculiar arrangement of the muscular fibres, which is not the same in the various species, and further to the fact that the system of the segmental nerves of the heart is in connexion with the nerves of the aorta.

The latter system is made up of some bundles travelling on the

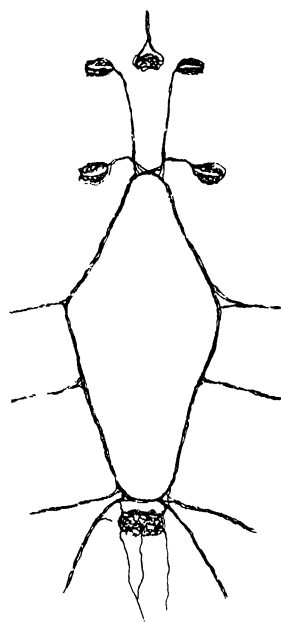
abdominal dorsal vessel and receiving in every segment branches from the abdominal nerves. I propose to call these branches *Nervi segmentales aortae* (Text-fig. 24, *Nn seg ao*). They supply the valves situated at the origin of the arteries. They supply the valves situated at the origin of the arteries



TEXT-FIG. 22.

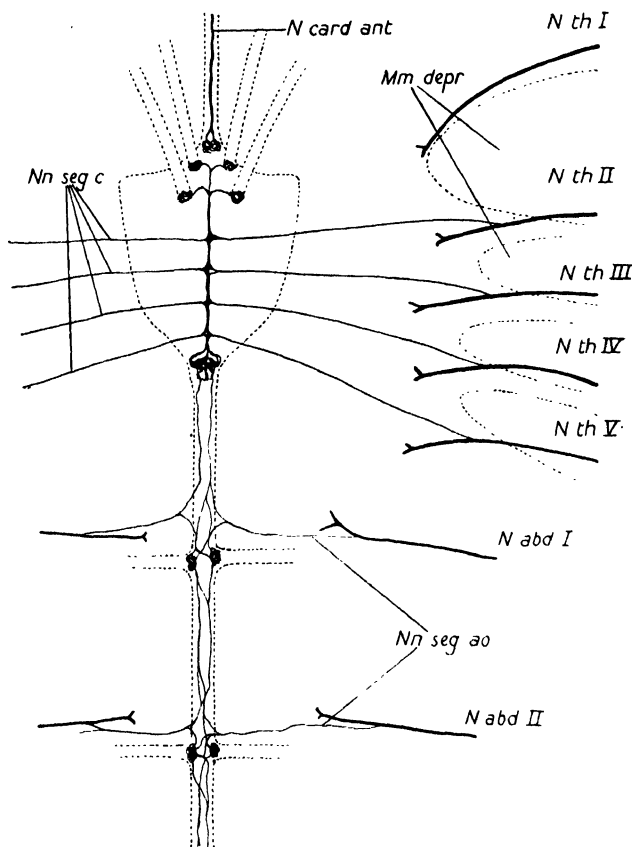
System of the nerves of the valves and of the muscles of the pericardium in *Scyllarus arctus*. Similar arrangement in *Homarus vulgaris* also.

The same as in fig. 22 in *Eriphia spinifrons*; similar arrangement in the other Crabs investigated. Figures 22 and 23, Pl. 15, should be compared with Text-fig. 2 representing this system in *Palinurus*.



TEXT-FIG. 23.

arising from the abdominal aorta. In the diagrammatic drawing (Text-fig. 24) the relations of the system of the segmental nerves of the heart and those of the aorta are shown. We note that the fibres springing from the 1st abdominal nerve take part in the



TEXT-FIG. 24.

Systems of the segmental nerves of the heart and of the segmental nerves of the abdominal aorta and their relations to the somatic nerves in *Potamobius astacus*. *Nn seg c*, nervi segmentales cordis; *N th I-V*, thoracic nerves; *Nn seg ao*, nervi segmentales aortae abdominalis; *N abd I, II*, abdominal nerves; *N card ant*, nervus cardiacus anterior (Nerve of Lemoine); *Mm depr*, depressor muscles.

innervation of the posterior valve of the heart; whether some from the segments farther back reach the heart too, or, vice versa, how far the fibres of the segmental nerves of the heart pass on the aorta, I am unable to give any exact information.

Turning to the nerves of the valves of the heart, we have to note that their appearance is somewhat different from that of the nerves described in previous sections. The fibrils are more uneven, the shape of their swellings (beads) is not the same. Even their colour when stained with methylene blue has often a different nuance.

On the periphery of the valve some fibres end in small leaf-like plates showing no connexions with the muscles. They may perhaps be regarded as the sensory endings. In the neighbourhood of the valves oblong swellings on the fibres were also observed. They could not be identified as the true cells.

It is a fact of the greatest interest that connexions could nowhere be found between the system of the segmental nerves and the other nervous elements of the heart. This statement must be accepted with some reserve owing to the difficulties of observing the elements of the neighbouring systems of fibres, the respective muscles being in close connexion with each other. However, it is easy to determine the limits between these two muscular systems because of the peculiar staining properties of the valvular muscles, for they very often become a distinct blue and are then sharply delimited from the muscles of the heart-wall (Text-fig. 25). Moreover, some details of the structure of the muscle-fibres of the valves are distinctive, since they are more loosely arranged and give off ramifying branches.

Attention may be called to the fact that these muscles, which are antagonistic to the muscles of the heart-wall, show a different behaviour when stained in methylene blue. This fact, which seems to prove that muscle-fibres in the same organ have various chemical or physico-chemical properties, may be of interest for the physiology of the involuntary (autonomic) system.

Notwithstanding a careful examination of all successful preparations I could find neither any irradiation of the local nervous system on the valves or of the nerves of the valves on the heart-wall, nor any anastomosis between the fibres of these two nervous systems.

Still more surprising is the fact that while the nerves of five valves are connected with each other, one valve only, namely,

that of the anterior median artery, is excluded from this common system and possesses its proper nerve.

2. *Nervus cardiacus anterior.*

There exists considerable bibliography about this nerve which was discovered by Lemoine in 1868 and called by him 'nerf cardiaque'. Its presence was asserted by several authors. In some recent works it was described as the main or even unique



TEXT-FIG. 25.

Microphotograph showing the muscles of the posterior arterial valve of the heart of *Potamobius astacus* deeply stained with methylene blue.

nerve of the heart. Accordingly, it was represented as giving stout branches in the heart which, as a matter of fact, do not exist. There is, however, to be noted the accuracy of the investigations of Police (1908), who confessed not to have succeeded in observing this branching, and stated that he only saw the nerve penetrate the wall of the artery: 'In vicinanza del cuore l'ho visto approfondarsi nella parete dell'arteria'. This observation is the most correct of all.

Police called this nerve 'nervus arteriosus medianus', and perhaps there is some reason for changing its name, but I have

preferred to adopt the term *nervus cardiacus anterior*, firstly because it has been known by the name '*nervus cardiacus*' for more than sixty years, and further, because, as a matter of fact, it reaches the heart at the origin of the artery. The name *nervi arteriosi* seems better reserved for the nerves of the artery in the abdomen. The addition of the adjective '*anterior*' to the old name seems necessary in order to distinguish this nerve from other nerves of the heart.

The anterior cardiac nerve arises from a nerve lying on the anterior and dorsal surface of the stomach (*nervus stomatogastricus*). For details concerning the complex innervation of oesophagus and stomach the reader may be referred to the works of E. J. Allen (1894), Police (1908), Keim (1915), and Orlov (1929). The cardiac nerve runs alongside the ophthalmic artery and, approaching the heart, divides into 2 or 3 branches (Text-figs. 2, 22, 23, 24). Staining with methylene blue reveals the destination of this nerve, viz. it penetrates the wall of the artery and breaks up into many fibres which supply the muscles of the median arterial valve. The appearance of these fibres, their branching, endings, and staining properties are the same as those of the nerves in other valves.

As already said, contrary to my expectation no communication with the latter could be found. Even if we suppose that some fibres escaped our observation, there might be only thin fibrils which certainly could not be got out by mere anatomical dissection. At any rate, the nerve of Lemoine, which ranked high in the innervation of the heart, must now be relegated to a much more modest place.

PHYSIOLOGICAL CONSIDERATIONS

In the foregoing description I touched in several places upon the probable functions of the nervous elements which had been found in the heart of the Crustacea. I shall now endeavour to summarize those points which may be of interest for physiology.

As to the local nervous system, its arrangement and relations with all the muscle-fibres of the heart lead to the conclusion that these muscles are under the influence of impulses originating in the ganglion cells of this system. The suggestion that the latter

may be sensory elements seems to me untenable, and I am strongly inclined to think that this system rules the beat of the heart.

Experimental evidence may be obtained with regard to the significance of the local system. Thus, in the heart of *Maia squinado*, though it is prepared for staining in the usual way, i.e. sectioned on its ventral side and stretched on the paraffin plate, the muscles, during one hour or more, continue to contract in a regular rhythm provided the ganglionic trunk be not damaged. But if now the latter be divided with fine scissors—this operation when the staining reveals the nerves may succeed without hurting the muscles—the heart stops beating at the very moment of the incision, and thenceforth only irregular contractions of various muscle-bundles take place. In cases in which, without this experimental incision, either such irregular contractions were observed or the heart did not beat at all, it could be ascertained that the ganglionic trunk or the main branches of it had been unintentionally injured when cutting the heart-wall.

It was tempting to make some experiments, also, on the cells themselves. But this is rendered difficult owing to the situation of the cells which, at least the anterior ones, lie under the muscles. At the section of the latter a part of the dendrites of the cells must be cut away and, moreover, it is almost impossible to work without hurting the ganglionic trunk.

From the above statements it would seem that the local system of ganglion cells in the Crustacea has the same function as, according to the investigations of Carlson, the corresponding system in the Xiphosura possesses. With regard to this question some experiments made by me when working on Isopoda may be of interest. In *Ligia oceanica* the ganglionic trunk is not covered by the muscles and, therefore, is more easily accessible. If it be divided by an incision, the heart continues to beat; but then each part of the heart containing one-half of the ganglionic trunk beats in a different, though regular, rhythm. It is even possible to cut the nervous trunk in two places, and then a separate rhythm of three portions of the heart may be perceived. On the contrary, if the heart itself be divided into

two, without cutting the nervous trunk, which is left like a bridge between the two parts, their beat remains synchronous (Alexandrowicz, 1931). The description of the nerves of the heart in Isopoda will be given in a subsequent publication.

Turning to the neurons of the local system in the heart of the Decapods, the problem to be solved is the function of the different projections of the cells. As for the long branches of the axons the preponderance of evidence indicates that the impulses are conveyed in them from the cells to the muscles. But what is the direction of the impulses in the short arborescent processes which arise both from the cell-body and from the axons? Are they transmitted from the cell to the periphery or in the opposite direction? As we have seen, these short processes also ramify in the muscles; would this not serve as an indication that they carry the impulses to the muscles, and, in consequence, that the cells of the local system possess several axons? We are, of course, accustomed to the schematic conception of nervous cells with one efferent fibre only, but there is no reason to assert that cells with several axons do not exist. Describing the innervation of the digestive organs in Cephalopods I suggested that the cells found there might have two or more efferent processes. Stöhr, in his recent work dealing with the histology of the involuntary nervous system, states that the distinguishing of the axon among the processes of the sympathetic ganglion cells has no sufficient base. Yet with regard to the neurons of the local system in the heart of the Crustacea we must take into consideration two facts: (1) the different aspect of endings of the short processes in the muscles when compared with the terminations of the long branches; and (2) the connexions of these arborescences with the efferent nerves. The first point, the peculiar histology of the terminations, suggests a different function from that of the motor endings of the long branches; the second, viz. the synapses with the efferent system, seems to indicate that the impulses in the short processes are carried from the periphery to the cells, if these impulses are to be thought of as destined for the effector parts of the neuron, i.e. for its axon.

I am aware that no direct evidence as to the course of the impulses can be brought forward, and, therefore, our conception

of these processes as dendrites is a hypothesis only, yet it seems to be more probable than any other.

Further, it may appear strange that the short arborescences springing from the axons are classified as dendrites like the processes originating from the cell-body. However, the presence of dendrites arising from the axon is known in Invertebrates and has been discussed recently in Hanström's work (1928). That in our case the proximal part of the axon is endowed with a particular function is suggested by the presence of the collaterals to the neuropile. In any case, as a peculiar feature of these neurons, we must take into consideration the presence of two kinds of dendrites, some arising from the cell-body, others from the axons, the latter at even a great distance from the cell.

Assuming then that the short arborescences are the dendrites which carry the impulses to the cells, we must search for the explanation of the function of their endings in the muscle-fibres themselves. We may imagine that the contraction of these fibres produces an excitation on the endings of the dendrites, and that resulting impulses are transmitted to the cell. Whatever kind of influence these impulses may exercise, whether they give rise to an excitation to be discharged in a following contraction of the muscles, or, on the contrary, have to inhibit some reaction in the cell during the diastolic period, at any rate, if our suggestion holds good, the impulses conveyed by the dendrites from the muscles would serve for the self-regulation of the rhythmical action of the neuromuscular apparatus of the heart.

The regulatory nerves (dorsal nerves) consist of fibres which, from the morphological point of view, were classified into two systems. The suggestion is obvious that one of these systems contains the inhibitory fibres whilst the other quickens the beat of the heart. However, to which of the two systems each of these regulating functions is to be ascribed I have but indirect indications. In the Stomatopoda I have found three separate pairs of nerves connecting the ganglionic trunk of the heart with the central nervous system. The most anterior pair carries inhibitory impulses, whereas the two posterior are

accelerator nerves. This fact is in accordance with the results of the investigations of Carlson, who had found the inhibitory nerves in Decapods originating in the infra-oesophageal ganglion in front of the roots of the accelerator fibres. Furthermore, in Stomatopoda the inhibitory fibres are of greater calibre than those of the accelerator fibres. All these data afforded us some basis for the suggestion that the thicker fibres in the dorsal nerves, i.e. what we have called the System I, have an inhibitory function. If that be the case, it would be directly shown that the inhibitory fibres are acting by synapses on the neurons of the local system. It may be recalled, further, that the terminal branches of these fibres are connected with each other by very numerous networks of fibrils. From this it may be concluded that this system acts as a whole—the impulses carried by the fibres being transmitted to a great number of terminal branches and from them transmitted to the neurons of the local system.

With regard to the system of the arterial valves and of the pericardium the following points may be of interest for physiology.

(1) The relations of the nerves of the valves to the nerves of the muscles of the pericardium.

(2) The absence of connexions¹ between the system of the valves and the local system.

(3) The absence of connexion between the nerves of the anterior median valve on the one hand and the system of the nerves of the remaining five valves on the other.

¹ I am not unaware that this statement, like similar assertions in general about the absence of nerve-fibres, has but relative value. Strictly speaking, it would be more correct to say everywhere that the connexions 'could not be found'. In investigations of the nervous system the cases are not rare when a successful preparation reveals at once a striking abundance of nervous elements in places where, even using special methods, nothing had been seen of them before. However, it must be pointed out that, in the cases under discussion, the absence of the nerve-fibres has a very high degree of probability owing to the fact that numerous preparations with well-stained nerves have been carefully examined as to this point.

These remarks may excuse the writer from repeating these restrictions when similar questions are under consideration.

The junction into one system of the nerves of the valves and those of the pericardium seems to be easily conceivable at first sight, having regard to the fact that the function of the muscles both of the valves and of the pericardium is antagonistic to that of the heart. When the latter is in systole the valves guarding the arteries must be open, while in the diastolic period they are closed and their muscles contracted. One might think that the muscles of the pericardium contract equally at each diastole and so the blood from the pericardial cavity is pressed into the heart. But this is not the case as has been already observed by Plateau (1880). Mangold (1925) states that although the pericardium is endowed with contractile properties the rhythm of its contractions is much slower than that of the heart. Observing this phenomenon in *Cancer pagurus* this writer found that one of these, as he called them, 'fluctuations of the tonus of the pericardium' falls from 27 to 80 beats of the heart. Thus we see that the same system of segmental nerves supplies the muscles of the valves and of the pericardium, working at a totally different rhythm. On the other hand the muscles of the valves, and those of the heart which contract, although alternately, at the same rhythm, do not show any connexion of their nervous apparatus. It is not impossible that this connexion goes by the long route of the central nervous system, but I venture to suggest the following explanation. Suppose the muscles of the valves to be in a tonic contraction as well as the muscles of the pericardium, both under the influence of impulses carried by the common system of the segmental nerves. Suppose further that the tonic contraction of the muscles in the valves may be relaxed in systole in response to some stimulus acting on the valves and produced by the blood itself. It may be that it is the pressure of the blood forcing its way, or, perhaps, there are some substances liberated in systole the action of which can relax the apparatus closing the valves. In any case the rhythmic sequence of the action of the valves and of the heart might be co-ordinated in this way without direct nervous connexions of the respective muscular apparatus. Admitting this interpretation the independence of the fibres supplying the valve of the anterior median artery would be of no decisive significance for the relaxation of

its muscles and the synchronous working with the valves of other arteries.

SUMMARY

The results of our investigations may be summarized as follows:

(1) In the heart of the Decapod Crustacea three systems of nervous elements can be distinguished, viz. (a) a local system of neurons which are distributed in the heart itself; (b) a system of fibres connecting the heart with the central nervous system; and (c) a system of nerves which supply the valves of the arteries arising from the heart as well as the muscles of the pericardium.

(2) The local nervous system consists of a nervous trunk situated in the dorsal wall of the heart near to its inner surface from which branches to the muscle-fibres of the heart are distributed. The main trunk is generally called the ganglionic trunk from the presence of nerve-cells in it. The cells are of two kinds: large and small. Their number was found to be constant, and comprises in *Cancer pagurus*, *Maia squinado*, and *Homarus vulgaris* the species which could be best investigated as to this point nine elements, viz. five large and four small cells. It seems that in other species of marine Decapods the number of cells in the hearts, if not the same, does not at any rate vary much. *Potamobius astacus*, however, has not less than sixteen elements (eight large, and eight, maybe, nine or ten, small ones). The cells are multipolar in shape. Their long processes—the axons—after sending out shorter ramifications run in regular courses, giving off long branches to all the muscles of the heart including those of the ostia, but excluding the muscle-fibres of the arterial valves. The short processes, which I regard as dendrites, spring from the cell-bodies and from the proximal parts of the axons. The endings of the dendrites which ramify in the muscle-bundles differ in appearance from the terminations of the long branches springing from the axons. The small cells possess similar processes, i.e. dendrites and axons. The latter could not be traced well.

In the main or ganglionic trunk which in different species has different shapes, the following elements are present: (a) large and small cells, the arrangement of which varies in different species, but in all cases the small cells are situated in the posterior part of the trunk; (b) the axons of the large and small cells and a part of their branches; (c) the fibres of the dorsal nerves; (d) the neuropile-like networks of fibrils where the synapses between the efferent fibres and the neurons of the local system take place. These neuropiles form several more compact masses in *Brachyura* whilst in *Macrura* they are more diffusely scattered in the ganglionic trunk.

(3) The fibres connecting the heart with the central nervous system take their origin in the infra-oesophageal ganglion and travel in the nerves running on the thoracic muscles. As separate bundles, one on each side, they turn towards the dorsal surface of the heart; hence the term *nervi cardiaci dorsales* is proposed; the other term—*regulator nerves*—indicates their physiological character. In their further course these nerves pierce the heart-wall and reach the local nervous system. The fibres of the dorsal nerves are of various diameters. The thicker, which in the description have been called System I, run throughout the ganglionic trunk and break up therein in many richly arborizing branches which at many places resemble in appearance the neuropile-like networks of fibrils. They are the fields of conjunction of all the fibres of System I with each other, as well as with the neurons of the local system. From the latter the following parts are in close relation with the fibrils of System I: (a) the collaterals of the axons entering the neuropile; (b) the dendrites; (c) the cell-bodies surrounded by a network of fibrils of the dorsal nerves; these basketworks, however, could not be seen in all the species investigated, and occur on the large cells only. Some branches of System I were found leaving the ganglionic trunk, but their destination is uncertain.

The remaining fibres of the dorsal nerves which, it may be assumed, do not belong to the System I are of smaller but not equal diameter. Some take their course to the muscles without entering the ganglionic trunk, others travel in the latter, but their distribution could not be made out.

(4) The third system of nerves, which enter into relationship with the heart by innervating the valves situated at the origin of the main arterial trunks, contains the following elements:

(a) *Nervi segmentales cordis*, which number, as was found in *Astacus*, four on each side, branch from the thoracic nerves and pass on the ventral side of the pericardium towards the middle line. Here they join into—according to the species—one or two bundles, which take a longitudinal course. From these bundles branches are given off to the valves of five arteries, viz. *arteriae antennales*, *arteriae hepaticae*, and *aorta posterior*, and to the muscles of the ventral pericardial plate. The latter receive also branches springing directly from the segmental nerves. The system of the segmental nerves of the heart is connected with the nerves of the dorsal abdominal artery which in its turn receives segmental nerves originating in the abdominal ganglia and ending in the valves of the arteries arising from the vessel named.

(b) The *nervus cardiacus anterior* arises from the stomatogastric nerve and runs alongside the median anterior artery. The territory of the terminal branches of this nerve, known as 'nerf cardiaque' of Lemoine, has been found to be confined entirely to the valve of the median anterior vessel (*aorta anterior* s. *arteria ophthalmica*). No connexion with the nerves of other valves could be ascertained.

(5) Besides the three nervous systems enumerated which are in relation with the heart itself, several nerve branches running from the thoracic nerves penetrate the pericardial cavity. They break up here in the neuropile-like networks situated on the so-called ligaments of the heart and on the connective tissue covering the dorsal wall of the heart.

(6) The probable function of all these elements is thought to be as follows: The local system is an 'autonomic' nervous apparatus from which the muscles of the heart receive impulses necessary for their regular contractions. The fact that the dendrites of the cells end in the muscles suggests that the rhythmical discharges in the nerve-cells are under the influence

of the rhythmical action of the muscles. Thus, there may be secured a reciprocal regulation of the process in two parts of the neuromuscular apparatus of the heart.

The dorsal nerves convey to the heart the inhibitory and accelerator fibres. Some evidence seems to indicate that the thicker fibres which have been found giving synapses with the neurons of the local system are endowed with the inhibitory function.

The nerves of the arterial valves may be considered as carrying impulses which hold the muscle-fibres in contraction during the diastolic period of the heart.

The nerves in the pericardial cavity, ending in fine networks, have evidently some sensory function.

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EXPLANATION OF PLATES 13, 14, AND 15.

PLATE 13.

Fig. 1.—*Maia squinado*. Large anterior cell. *ax*, axon.

Fig. 2.—*Maia squinado*. Part of larger neuropile. *nd*, fibre of the dorsal nerve; *ax*, axon of a large cell. In this preparation the axon deviates from the usual course and owing to this accidental position its collaterals to the neuropile are distinctly seen.

Fig. 3.—*Cancer pagurus*. Large cell with the pericellular network and accessory fibres (*ac*); *ax*, axon.

Fig. 4.—*Cancer pagurus*. Branching of two arborescences of a large cell in the same muscle bundle. *ac*, accessory fibres; *ax*, axon.

Fig. 5.—*Maia squinado*. Two small cells. *ax*, their axons; *col*, collaterals to the small neuropiles.

Fig. 6.—*Maia squinado*. Small cell. *ax*, axon; *nd*, two fibres of the dorsal nerves. These fibres give off collaterals to the neuropiles and branches which accompany the dendrites.

Fig. 7.—*Palinurus vulgaris*. Dorsal nerve entering the heart at the point indicated by the line *a-a*. The nerves are represented as if the heart-wall were totally transparent. *S II*, fibre of System II branching on

the inner surface of the heart; *np*, nerves on the exterior surface of the heart in the pericardial cavity, breaking up in a dense 'punctate substance'.

PLATE 14.

MICROPHOTOGRAPHS.

Fig. 8.—*Maia squinado*. Anterior part of the ganglionic trunk. The three anterior cells are distinctly seen.

Fig. 9.—*Palinurus vulgaris*. Anterior part of the ganglionic trunk. The anterior cells and one situated farther back are distinguishable (*gc*).

Fig. 10.—*Munida rugosa*. Ganglionic trunk. *gc*, ganglion cells.

Fig. 11.—*Maia squinado*. Posterior part of the ganglionic trunk. Two large and four small cells are seen. The position of one of the large cells which lies forwards from the bifurcation of the trunk is not the typical one.

Fig. 12.—*Cancer pagurus*. Anterior bifurcation of the ganglionic trunk. *gc*, large cell with a thick dendrite running laterally; the ramifications of this dendrite which lie deeper in the muscles could not be represented; *ax*, *ax*, axons of the large lateral cells; *nd*, fibres of the dorsal nerves (cf. Text-fig. 18); *a*₁, thick fibre running forwards belonging to axon *a*₁ of the diagram, Text-fig. 8, but in the preparation represented in the microphotograph this fibre runs nearer to the median line than in the diagram.

Fig. 13.—*Cancer pagurus*. Posterior part of the ganglionic trunk. Four small cells deeply stained, with several projections. *gc*, large cell weakly stained, with several fibres surrounding the cell-body; *nd*, fibre of the dorsal nerve.

Fig. 14.—*Homarus vulgaris*. Short arborescence originating with two branches in the neighbourhood of the anterior bifurcation (*bif*) of the ganglionic trunk. *gc*, large cell insufficiently stained.

Fig. 15.—*Homarus vulgaris*. Dendrite of the large cell ramifying in several thick branches.

PLATE 15.

MICROPHOTOGRAPHS.

Fig. 16.—*Palinurus vulgaris*. Branching of the axon (*ax*) of a large cell running down the ganglionic trunk; the respective cell is not included in this figure; *nd*, fibre of the dorsal nerve.

Fig. 17.—*Palinurus vulgaris*. Dendrites arising from the axon of small cells.

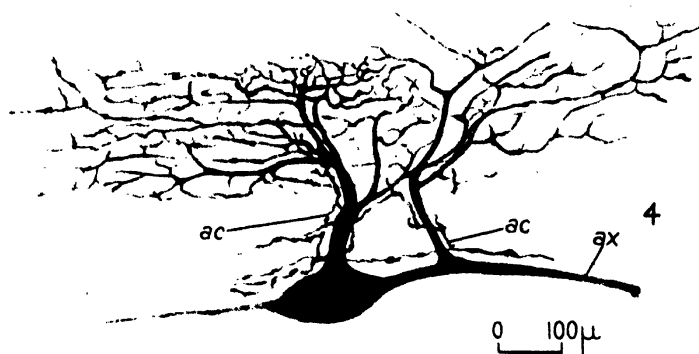
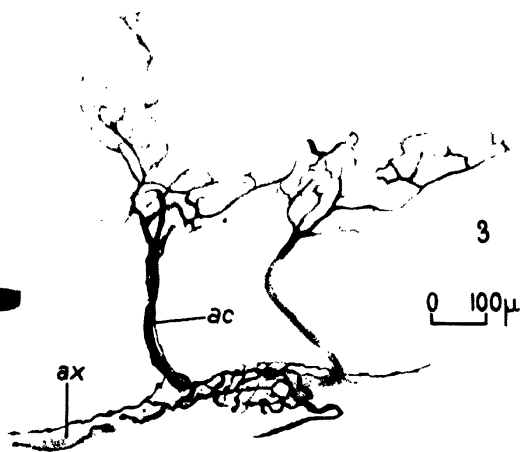
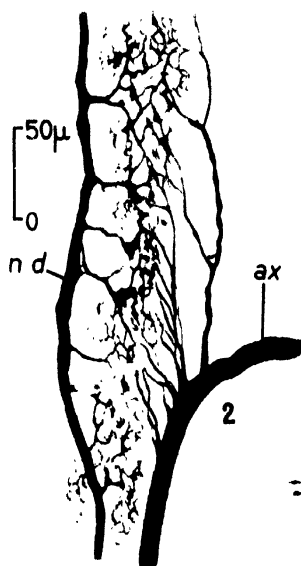
Figs. 18, 19.—*Cancer pagurus*. Parts of the circular trunks at the point of branching of the antero-lateral and lateral nerves; five axons of the circular trunk are distinctly seen (cf. Text-figs. 4 and 8).

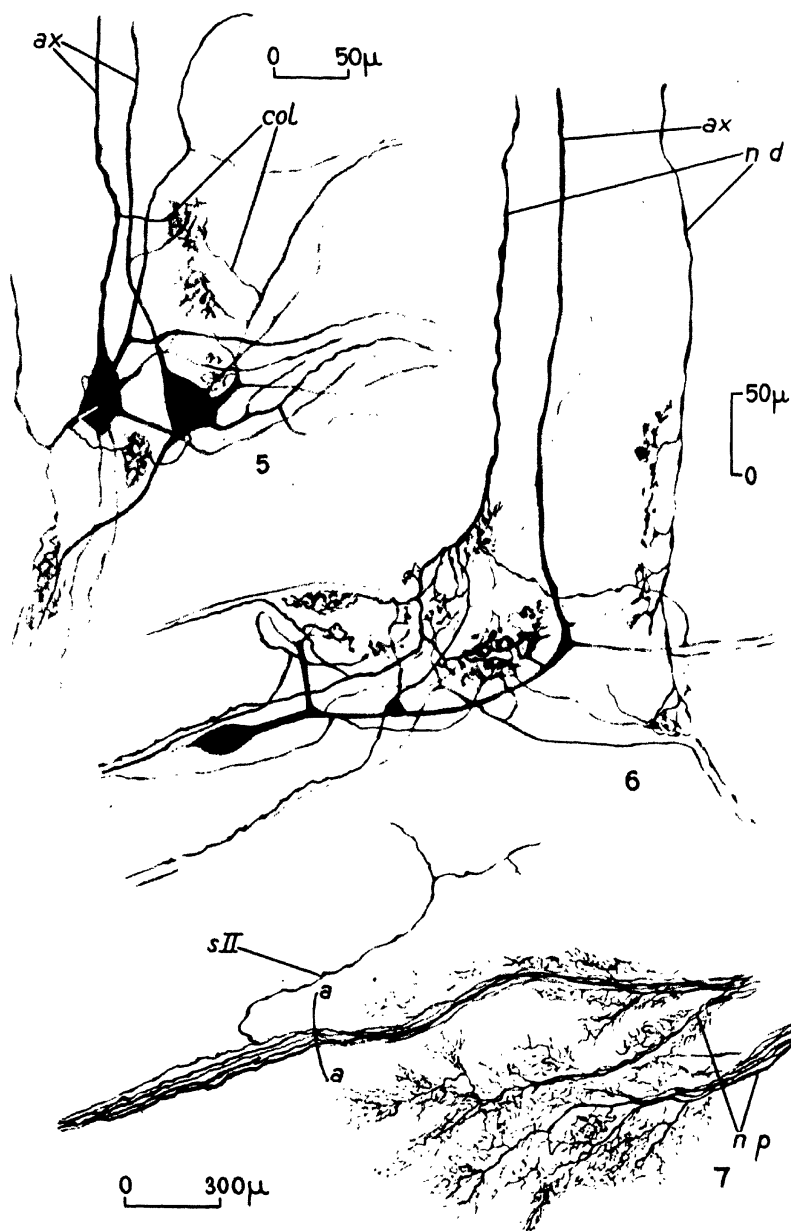
Fig. 20.—*Palinurus vulgaris*. Two large cells surrounded by thin fibres originating in the dorsal nerves. One of the cells, lying deeper, could not be sharply focused.

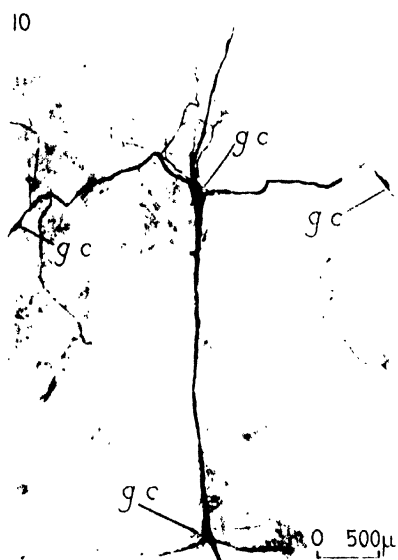
Fig. 21.—*Palinurus vulgaris*. Ramifications of two fibres of the dorsal nerves (*nd*) entering the ganglionic trunk. *d*, *b*, short branches arising from these fibres and running laterally from the ganglionic trunk; their formation of several thin fibres is well seen (especially in *d*).

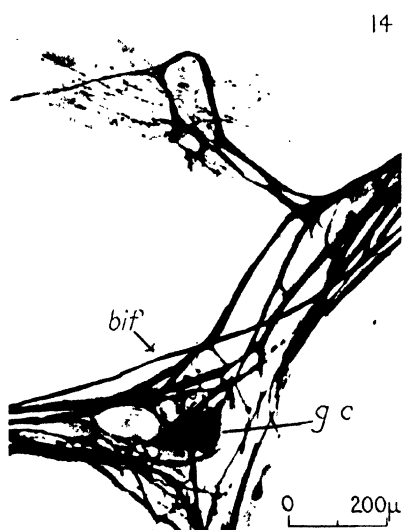
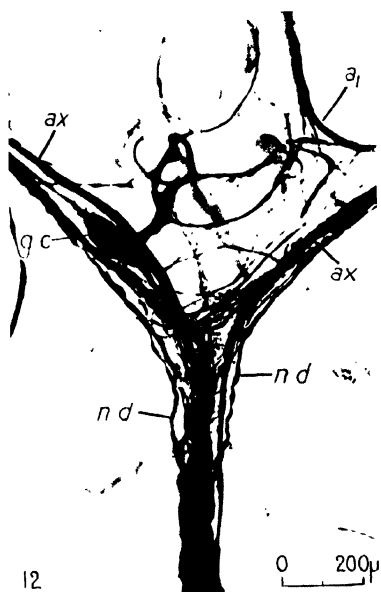
Fig. 22.—*Palinurus vulgaris*. A fragment of the fasciculus longitudinalis pericardii (cf. Text-fig. 2).

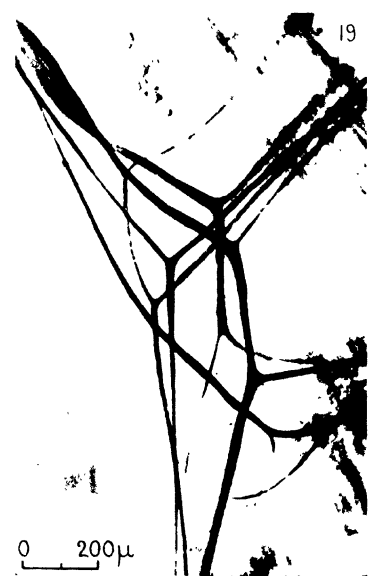
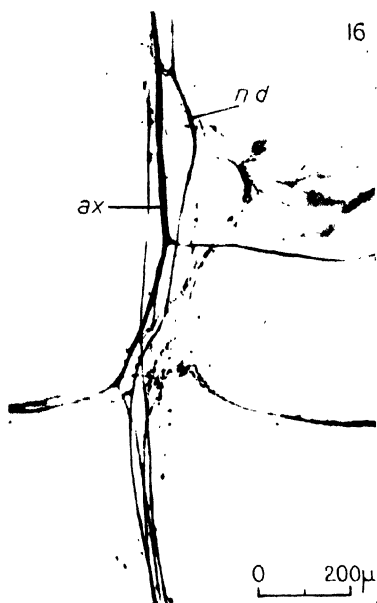
Fig. 23.—Same preparation as fig. 22. Ramification of the nerves in the pericardial muscles (cf. Text-fig. 2).











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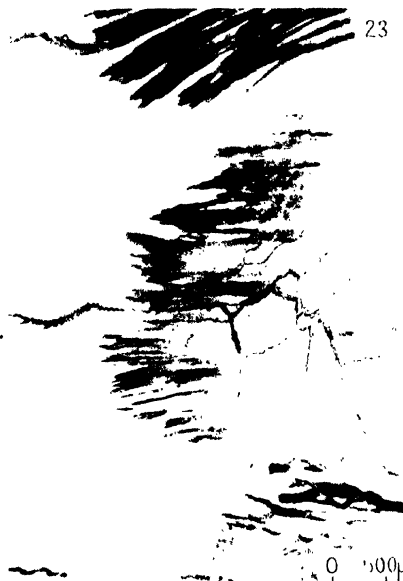
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of the breeding habits of the tuatara the eggs are laid in November and take fourteen or fifteen months to hatch. Development proceeds rapidly at first but slows up in March, at the approach of winter, and remains practically at a standstill until the following summer. The young appear to hatch in the middle of the summer (January). The period elapsing between laying and hatching is thus extraordinarily long and is probably unique among oviparous vertebrates. The embryos available form an extremely fine series which Dendy divided into sixteen stages distinguished by the letters C to S. Some idea of the rate of development of the successive stages can be obtained from the following tabular summary of the dates at which they were obtained.

TABLE.

Ovarian eggs (ready for laying)	November 1st.
Stage C	„ 22nd.
„ D	December 9th.
„ E	„ „
„ F	„ 9th to 10th.
„ G	„ 9th.
„ H	„ 16th.
„ J	„ 9th to 16th.
„ K	„ „
„ L	„ 16th to 27th.
„ M	December 27th to January 3rd.
„ N	January 6th.
„ O	„ 6th to 25th.
„ P	„ 25th.
„ Q	„ (end).
„ R	March 8th to July (end).
„ S	January 6th.

It is not proposed, in the present paper, to give more than a brief account of the more outstanding features which distinguish each stage and which should serve as a sufficient basis for the description of the origin and migration of the primordial germ-cells.

These descriptions are based as far as possible on original

observations, but much use has been made also of Dendy's descriptions. A more detailed account of the stages described is to be found in Dendy's original paper (4) to which reference should be made. The letter indicating the stage and the number assigned to the embryo by him will be used throughout this paper.

The material was fixed for the most part in Kleinenberg's picric acid, and the majority of the embryos were stained in borax carmine. Some of the embryos, however, were stained with Ehrlich's haematoxylin.

DESCRIPTION OF STAGES.

Stage C.

(One embryo, C' 9, cut longitudinally. Fig. 1. Pl. 16.)

The blastoderm in this, the earliest stage obtained, already extends completely around the yolk, the ectoderm is composed of a single layer of cells, and immediately beneath is a loose network of stellate cells lying in the outermost part of the yolk (Dendy). The primitive knot is situated at the hinder end of the embryonal shield. The blastoporic depression is bounded in front by a well-marked dorsal lip and behind by the undifferentiated primitive knot tissue. From the hinder surface of the knot tissue the ectoderm continues away back as a thin superficial layer, the endoderm as a thick layer beneath the ectoderm which passes over behind into the yolk-containing germ-wall (primitive yolk-sac endoderm). Continued forwards from the blastoporic depression is a thick tapering mass of cells representing the head process.

Primordial germ-cells are present but few even in this early stage; others are in process of differentiation. They occur in the yolk-sac endoderm of the area opaca all around the embryo (fig. 1, Pl. 16). The yolk-sac endoderm of this region is composed of a loose meshwork of stellate cells of various sizes, in and between which large yolk-spheres are plentifully scattered. The yolk-spheres are undergoing rapid absorption and are breaking up in the endoderm cells into granules of various sizes. The primordial germ-cells are distinguishable from the other cells of the yolk-sac endoderm by their large size and by their rich

content of yolk-granules, all of which are approximately equal in size. Apparently these characteristic granules of yolk are formed by the breaking down of larger yolk-spheres within the developing primordial germ-cells. The primordial cells in these early stages often have cytoplasmic processes which give them a stellate shape. When fully formed they are spherical or ovate in shape and are considerably larger than any of the other cells of the endoderm. The fully formed primordial germ-cells have no long processes, but sometimes have short, blunt processes which is in accord with their probable amoeboid character. Thus, their most remarkable characteristics are the extraordinarily early stage at which they are differentiated, their large size in comparison with any of the other cells of the blastoderm, and their distinctive content of yolk-granules.

Stage D.

(One embryo, D 58, cut transversely. Text-figs. 1 and 2.)

The embryonal shield in this stage is longer and is oval. No trace of an amnion is yet visible, but the head-fold of the embryo is well marked and the fore-gut lies within it. A short, possibly discontinuous, chorda-canal (archenteron) runs obliquely forwards and downward from the blastopore and possibly opens below into the sub-germinal cavity. The notochord is formed, as also is the paraxial mesoderm on either side of it, whilst mesoderm is in process of rapid proliferation from the primitive knot region. The mesoderm has extended out well beyond the limits of the embryo. The medullary plate is well marked and is grooved. Blood-islands are beginning to make their appearance at the inner limit of the area opaca.

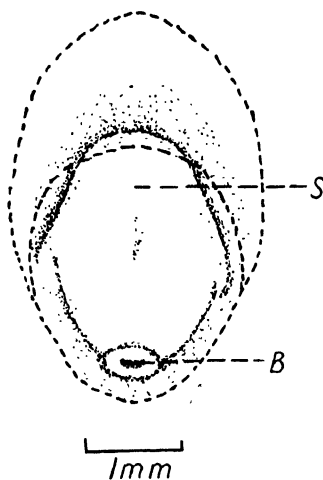
The primordial germ-cells are present in this stage in much larger numbers than in the preceding and are still in process of formation. They are still present only in the yolk-sac endoderm. Cranially they occur beneath the head and in front of it, laterally they are situated close to the embryo and extend back as far as the primitive knot. They are much more numerous beneath and in front of the head than elsewhere (Text-figs. 1 and 2). They can be observed in process of formation, as described in stage C, in the yolk-sac endoderm just cranial to the head.

Stage E.

(Two embryos, E 56 and E 64, both cut transversely.

Fig. 2, Pl. 16, and Text-figs. 3 and 4.)

This stage shows a considerable advance on the preceding in that the amnion is now present.¹ The head of the embryo is



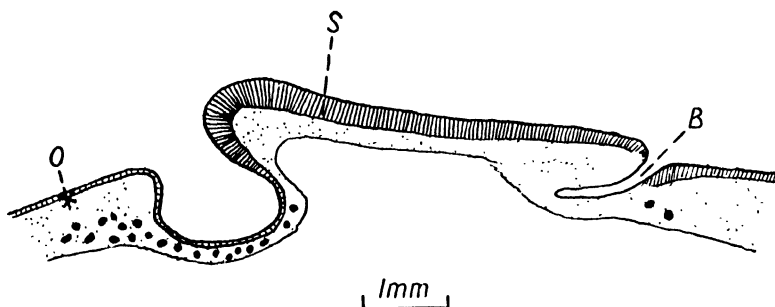
TEXT-FIG. 1.

Diagram of the blastoderm of stage D from above. The primordial germ-cells are distributed between the dotted lines anteriorly and around the single dotted line posteriorly. *B*, blastopore; *S*, embryonal shield (modified from Dendy).

completely invested by the proamnion, which latter is continued back by the double-layered ectodermal primordium of the amnion. The amniotic tube opens at the level of the caudal limit of the embryo. There are, as yet, no somites. In one embryo the chorda-canal (neurenteric canal) runs inwards from the medullary groove and takes a short course through the primitive knot tissue, but its opening into the sub-germinal cavity is occluded. A thin membrane-like layer forms the floor of the sub-germinal cavity. Differentiated primordial

¹ A paper, by one of the authors (M. T.), on the development of the amnion in *Sphenodon* is in preparation.

germ-cells are still more numerous in the embryos of this stage than in stage D. They occur in considerable numbers in the yolk-sac endoderm of the area pellucida in front of and lateral to the head (Text-fig. 3). Others are to be found in various stages of differentiation in the yolk-sac endoderm of the area opaca (fig. 2, Pl. 16) close to the margin of the area pellucida, and a few also in the yolk just beneath the membrane which forms the floor of the sub-germinal cavity. A number of primordial germ-cells are also present lateral to the region of the primitive knot. A number of primordial germ-cells are also present lateral to the region of the primitive knot



TEXT-FIG. 2.

Diagrammatic median sagittal section of stage D, reconstructed from the transverse series. The primordial germ-cells, shown as large black dots, are distributed in the yolk-sac endoderm below and in front of the head of the embryo and in smaller numbers in the yolk-sac endoderm just behind the primitive knot. *B*, blastopore; *O*, bilaminar blastoderm; *S*, embryonal shield.

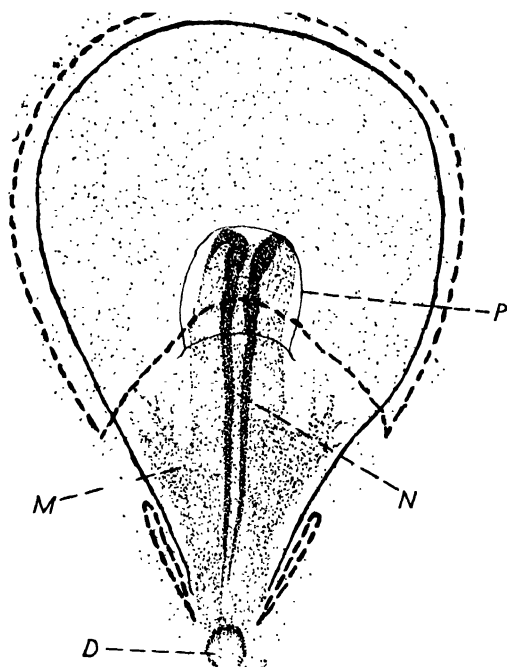
(streak) (Text-fig. 3). They form a strand-like structure on each side close to the lateral margin of the sub-germinal cavity in the yolk-sac endoderm at the point where that of the area pellucida merges with that of the area opaca (germ-wall). This cluster of primordial germs-cells (Text-fig. 4) is particularly striking in embryo E 64.

Stage F.

(Two embryos, F 61, cut longitudinally, and F 72, cut transversely. Text-fig. 5.)

The posterior opening of the amniotic tube is now situated some considerable distance behind the embryo, the posterior

amniotic canal being about half as long as the embryo itself. Lateral pleuro-pericardial cavities are now present and the primordia of the myocardium and endocardium of the heart.



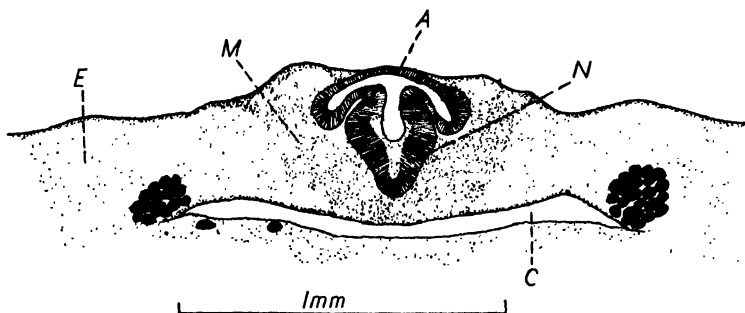
TEXT-FIG. 3.

Diagram of the blastoderm of stage E from above. The areas of distribution of the primordial germ-cells are enclosed in dotted lines. The area pellucida is enclosed in the continuous line. *D*, posterior amniotic opening; *M*, mesoderm; *N*, medullary tube; *P*, proamnion (modified from Dendy).

The neurenteric canal, leading from the cavity of the amnion, opens below in one embryo into the sub-germinal cavity.

Primordial germ-cells are not being formed so far as can be determined from the material. Since only the blastoderm in the vicinity of the embryo is preserved it is impossible to say whether

or not they are still being formed in the more remote parts. The majority of the primordial germ-cells are situated in front of and lateral to the head (Text-fig. 5). They are localized in the yolk-sac endoderm, the majority occurring in the thick endoderm of the area opaca where it joins the thin endoderm of the area pellucida, but a few are found in the latter around its



TEXT-FIG. 4.

Diagrammatic transverse section just in front of the neurenteric canal of stage E. The primordial germ-cells, shown as large black dots, are in a cluster at each side at the edge of the sub-germinal cavity. A few are present in the floor of the sub-germinal cavity. *A*, ectodermal amnion; *C*, sub-germinal cavity; *E*, yolk-sac endoderm; *M*, mesoderm; *N*, medullary tube.

margin. No primordial germ-cells are present in the embryo itself, or in the proamnion, or in the yolk-sac endoderm immediately overlying the head of the embryo. The primordial germ-cells are thus distributed in a crescent round the head. This distribution is similar to that found in stage E, but is more clearly defined. Primordial germ-cells are also present in smaller numbers in the yolk-sac endoderm lateral to the embryo and immediately behind the primitive knot (streak), thus continuing the tips of the crescent around the head and completing the ring of primordial germ-cells around the embryo. Those primordial germ-cells which are posterior to the primitive knot in both embryos are situated in the yolk-sac endoderm touching the primitive knot; one or two of them appear to be partly in the substance of the primitive knot itself.

Stage G.

(One embryo, G 59, cut longitudinally.)

The amniotic tube in this embryo opens to the exterior at the level of the primitive knot (streak). There are probably about four pairs of somites. The medullary tube is closed throughout its length except for the anterior neuropore. A neurenteric



TEXT-FIG. 5.

Diagrammatic longitudinal section of stage F. The primordial germ-cells from thirty-two adjacent sections are superimposed and are shown as large black dots. The majority are in front of the embryo in the yolk-sac endoderm at the junction of the area pellucida and the area opaca. A few are in the yolk-sac endoderm just within and immediately behind the primitive knot (streak). *A*, ectodermal amnion; *D*, posterior amniotic opening; *E*, yolk-sac endoderm; *K*, primitive knot (streak) (modified from Dendy).

canal is present, leading from the amniotic cavity through the medullary tube to the sub-germinal cavity.

The single specimen of this stage has so little of the extra-embryonal tissues attached that it is impossible to describe the distribution of the primordial germ-cells beyond stating that many are present in the yolk-sac endoderm of the area pellucida in front of and lateral to the head. Several primordial germ-cells are present, as in the previous stage, in the endoderm in the immediate vicinity of the primitive knot (streak). Primordial germ-cells are still entirely absent from the embryonal tissues.

Stage H.

Marked increase in the length of the fore-gut has taken place. An oral plate is present. The heart is conspicuous as a pear-shaped sac, and the endocardial heart tubes are fused for some distance. Vitelline veins are present. There are at least twelve pairs of somites (embryo damaged). Primary optic vesicles are

in process of being constricted off from the fore-brain. Neither hind-brain nor spinal cord is closed, and it is possible that this fact, taken in conjunction with the further fact that the head is quite straight, points to the conclusion that this embryo is not altogether normal.

The single embryo of this stage is so imperfect that it is impossible to define the distribution of the primordial germ-cells. Those that are present in the vicinity of the embryo conform to the distribution observed in stages F and G, except that a very few are situated in the endoderm of the yolk-sac of the area pellucida immediately above the head, as well as in front of and lateral to the head, and one is present in the sero-amniotic connexion. No primordial germ-cells are present in the embryonal tissues themselves.

Stage J.

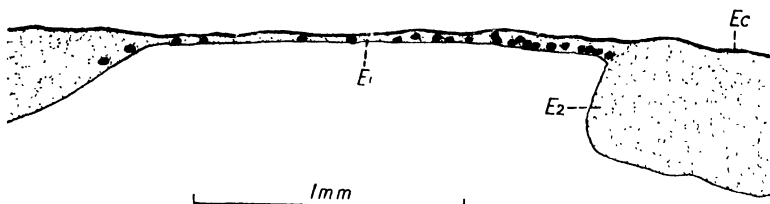
(Three embryos, J 44, J 46, and J 79, cut transversely.

Text-figs. 6, 7, and 8.)

This important stage is represented by a series of embryos. Reference will be made to one embryo only, except where variation occurs. The posterior amniotic canal is still conspicuous. The cephalic flexure is well marked and the proamnion is formed as far back as the anterior intestinal portal. The first gill-pouch is only separated from the exterior by its limiting membrane. In one embryo a short hind-gut is present. Dendy observed the heart to be beating slowly in one living embryo. The heart has the form of a wide tube slightly bent on itself, but the fusion of the endocardial tubes is still incomplete. One aortic arch is present. The vitelline arteries are now formed. The sinus terminalis encircles the hinder two-thirds of the embryo. There are two pairs of nephric vesicles, the Wolffian duct ending blindly behind. There are fourteen pairs of somites. The anterior neuropore still persists. A neurenteric canal leads from the medullary tube into the sub-germinal cavity. Auditory pits, still widely open, are present.

Primordial germ-cells occur in considerable numbers in the yolk-sac endoderm in front of (Text-fig. 6) and lateral to the

head, as in previous stages. They are now also present above the head (Text-fig. 7) as far back as the level of the junction of the proamnion with the yolk-sac endoderm of the bilaminar blastoderm. They occur chiefly in the endoderm of the area pellucida, but also in the thicker endoderm of the area opaca close to the margin of the area pellucida. Behind the proamnion the primordial germ-cells occur only laterally to the embryo, frequently in small clumps, and in the yolk-sac mesoderm as well as in the yolk-sac endoderm. Where the yolk-sac



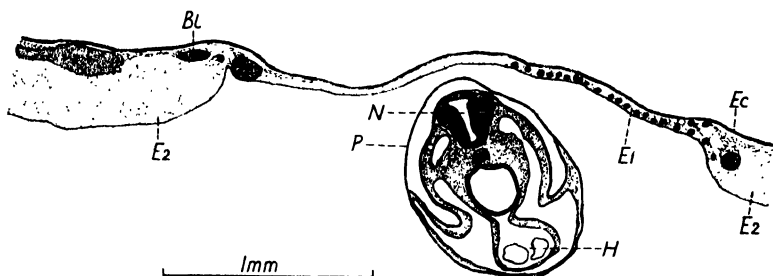
TEXT-FIG. 6.

Diagrammatic transverse section through the bilaminar blastoderm in front of the head in stage J. The primordial germ-cells, shown as large black dots, are almost entirely confined to the endoderm of the area pellucida. *Ec*, ectoderm; *E1*, endoderm of area pellucida; *E2*, endoderm of area opaca.

mesoderm is split by the coelom the primordial germ-cells occur in the splanchnic mesoderm and endoderm (Text-fig. 8) and not in the somatopleure. Primordial germ-cells occur more frequently in the sinus terminalis, which is solid at this stage, and in the blood-islands, than elsewhere in the mesoderm. A very few are present just behind the embryo in the mesoderm of the yolk-sac splanchnopleure.

The primordial germ-cells in the yolk-sac splanchnopleure in the region just behind and lateral to the heart are thus in close relation to the network of vitelline blood-vessels. Embryo J 46 differs from both the other embryos of this stage and from all the earlier stages in that a number of the primordial germ-cells are found actually in the vessels in this region (Text-fig. 8). Those that are in the vessels are in factors of the vitelline veins, when near the embryo; or in common plexuses of vitelline veins and vitelline arteries, when farther from the embryo.

It will be seen, in comparison with later stages, that the primordial germ-cells are now beginning to migrate from the yolk-sac endoderm (in which they were formed) into the embryo. This migration appears to be effected partly by their own amoeboid movement and partly passively in the venous blood-stream. Those primordial germ-cells that are situated in the yolk-sac mesoderm and endoderm of the bilaminar blastoderm in front, above and lateral to the head, migrate outwards and backwards



TEXT-FIG. 7.

Diagrammatic transverse section through the embryo of stage J at the level of the heart. The primordial germ-cells from twenty-two adjacent sections are superimposed and are shown as large black dots. They are numerous in the endoderm of the bilaminar blastoderm of the area pellucida above and lateral to the embryo. They also occur in the blood-islands at the margin of the area pellucida. *Bl*, blood-island; *Ec*, ectoderm; *E*¹, endoderm of area pellucida; *E*², endoderm of area opaca; *H*, heart; *N*, medullary tube; *P*, proamnion.

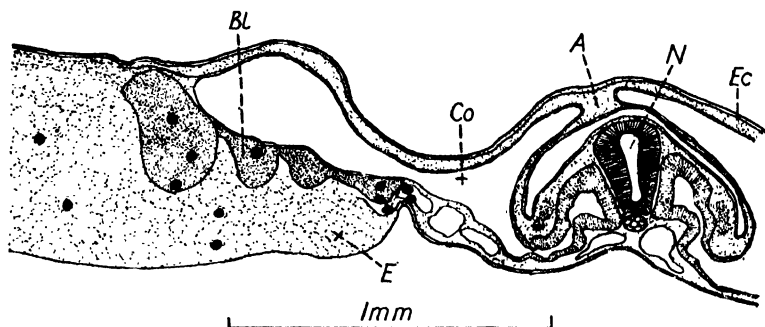
into the yolk-sac splanchnopleure, at the level of the junction of the proamnion with the ectodermal amnion (cf. Text-figs. 7 and 8). Once in the yolk-sac splanchnopleure of this region, they travel inwards towards the embryo, either in the mesoderm or endoderm, and may sooner or later penetrate a vessel and so get carried into the embryo with the venous blood.

Stage K.

(One embryo, K 39, cut transversely. Fig. 9, Pl. 17, and Text-fig. 9.)

The posterior amniotic canal has virtually disappeared. The oral plate is perforated, and the first pair of gill-pouches opens

to the exterior in one embryo. The thyroid primordium is recognizable. A small endodermal allantoic outgrowth is present. The heart was observed to be beating, here, as in J (Dendy). Cardinal and umbilical vessels are present. There are about twenty-three pairs of somites. The secondary optic vesicles are in process of formation and the lens thickenings are recognizable. The pineal eye primordium appears as a small



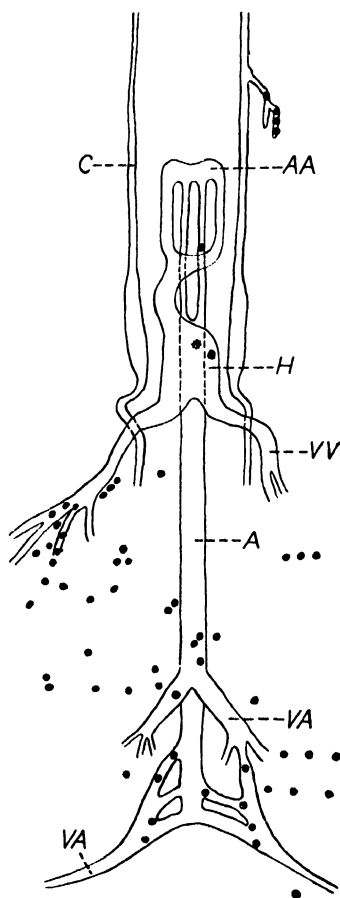
TEXT-FIG. 8.

Diagrammatic transverse section through the trunk of the embryo of stage J. The primordial germ-cells from twenty-eight adjacent sections are superimposed and are shown as large black dots. They occur in the extra-embryonal splanchnic mesoderm and endoderm, especially in the sinus terminalis. *A*, ectodermal amnion; *BL*, blood-island; *Co*, coelom; *E*, extra-embryonal splanchnic endoderm; *Ec*, extra-embryonal ectoderm; *N*, medullary tube.

round diverticulum from the fore-brain. The neurenteric canal opens below into the hind-gut.

This embryo was sectioned with very little of the neighbouring blastoderm attached. There is none whatever attached to the anterior region and only a small amount on each side posteriorly to the level of the heart. A detached portion of the blastoderm, which was probably originally situated cranially to the head, is present, and contains many primordial germ-cells in the splanchnic mesoderm and endoderm. Behind the level of the heart many primordial germ-cells are found in the extra-embryonal splanchnic mesoderm and endoderm close to the embryo in the vicinity of the vitelline veins and vitelline arteries.

A very few are found behind the vitelline arteries and only one posterior to the embryo in the extra-embryonic splanchnic



TEXT-FIG. 9.

Diagrammatic plan of the vascular system of stage K. The primordial germ-cells are shown as large black dots. *AA*, aortic arch; *A*, dorsal aorta; *C*, cardinal vein; *H*, heart; *VA*, vitelline artery; *VV*, vitelline vein.

mesoderm. The primordial germ-cells in the extra-embryonic splanchnic mesoderm are most plentiful in the vicinity of the

vitelline veins in front, and of the vitelline arteries behind, and many of them are located in blood-islands or in the veins or arteries themselves.

This stage differs from all the earlier stages in that a number of primordial germ-cells occur within the embryo itself, chiefly in the blood-vessels (Text-fig. 9). Some are found in the factors of the vitelline veins (fig. 9, Pl. 17), one is present in the heart, one in the dorsal portion of one of the single pair of aortic arches, and one in the dorsal aorta at the level of the heart. Four are present in the head itself in the small vessels, dorsal and ventral to the optic stalk, which apparently communicate with the anterior cardinal vein. Behind the level of the anterior intestinal portal a number of primordial germ-cells occur in the aorta and vitelline arteries and in the splanchnic mesoderm close to them. One primordial germ-cell appears to be free in the coelom in the lumen of one of the nephric funnels at the level of the anterior intestinal portal. This distribution within the embryo can only be accounted for by assuming that the primordial germ-cells, entering the factors of the vitelline veins, get carried into the body in the circulation and, travelling *via* the heart and aortic arches, arrive in the aorta at the region of the future germinal ridges. There some of them pass through the walls of the aorta and wander in the tissues of the splanchnopleure. Others appear to get carried on in the arterial blood and to pass out of the embryo again in the vitelline arteries; they would presumably get into the venous blood-stream and so gain access to the embryo again. The occasional primordial germ-cells found in the head of this embryo and in those of later stages appear to have got jammed in the small vessels and would probably degenerate.

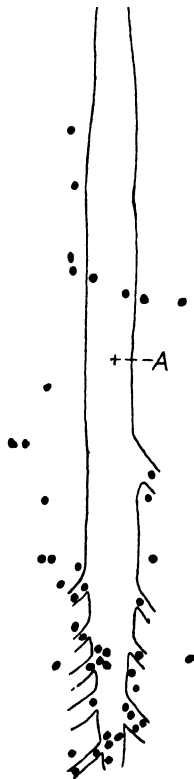
Stage L.

(Two embryos, L 50, cut obliquely and broken, and L 47, mounted whole. Fig. 7, Pl. 17, and Text-figs. 10 and 11.)

The cervical flexure is now present, and a short tail. Fore and hind limb buds are present. Three visceral pouches now open to the exterior. The allantois is visible externally as a finger-shaped outgrowth. The Wolffian ducts open into the cloaca. Tail gut

is present. There are thirty somites at least. The primordia of the cerebral hemispheres are recognizable. The auditory pits are almost closed. Nasal pits are present. The neurenteric canal is still present.

Primordial germ-cells are still present lateral to the embryo in



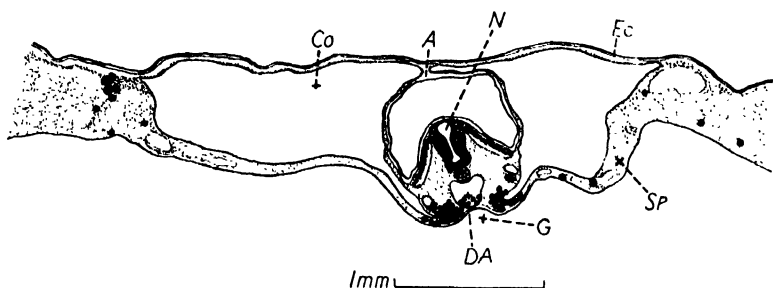
TEXT-FIG. 10.

Diagrammatic plan of the dorsal aorta and neighbouring germ-cells, the later shown as large black dots, of stage L. A, dorsal aorta.

the extra-embryonal splanchnopleure. The number of primordial germ-cells found outside the embryo is, however, greatly reduced, and those that are present are chiefly near the embryo.

The number of primordial germ-cells found within the embryo has increased greatly in comparison with the previous stage.

Thirty-eight primordial germ-cells are found in the head of embryo L 50 alone. These are situated chiefly in the mesenchyme or small vessels below the floor of the fore-brain and around the optic vesicle. The majority of the primordial germ-cells found within the embryo are in the region of the developing mesonephros and germinal ridges. They are present in considerable numbers in the aorta (Text-fig. 10) and the vitelline arteries arising from it in this region, as well as in the mesoderm immediately below and lateral to the aorta (Text-fig. 11). Many are present already in the primordia of the germinal ridges them-



TEXT-FIG. 11.

Diagrammatic transverse section of the embryo of stage L, at the level of the mesonephros. The primordial germ-cells, shown as large black dots, are clustered in the germinal ridges, and in their vicinity in the aorta itself, and in the mesoderm bounding the mid-gut groove. Others are present in the mesoderm of the extra-embryonal splanchnopleure and in the mesoderm and endoderm of the trilaminar yolk-sac wall beyond the limit of the coelom. *A*, ectodermal amnion; *Co*, coelom; *DA*, dorsal aorta; *Ec*, ectoderm; *G*, mid-gut groove; *N*, medullary tube; *Sp*, splanchnic mesoderm.

selves. Many others are present close to the germinal ridges in the mesoderm bounding the deep mid-gut groove (fig. 7, Pl. 17) in this region or in small vessels in it. The primordial germ-cells thus appear to be following two distinct routes to the germinal ridges. One route is *via* the aorta and possibly the mesonephric arteries; the other is through the mesoderm bounding the mid-gut groove.

It is an important fact that, whilst the primordial germ-cells have arrived in this stage at the site of the future gonads, the

germinal ridges as such are not differentiated in any other way. The region in which each will form is clearly delimited by the mesentery of the mid-gut on one side and by the forming mesonephros on the other, but the peritoneal epithelium covering this region does not exhibit the characteristic thickening which results in the formation of the germinal ridge.

Stage M.

(Two embryos, M 51 and M 81, cut transversely.)

The anterior half of the embryo is enclosed in the proamnion. The cervical flexure is more marked. Fore and hind limb buds are conspicuous. The tail is longer and its spiral twist is more marked. There are five visceral pouches, three of which open to the exterior. The liver is now conspicuous externally. The allantois has increased considerably in size and is now vascularized. The Wolffian ducts, unlike those of the preceding embryo, do not open into the cloaca. A proctodaeal pit is probably present. There are (in one embryo) forty-one somites. The lens of the eye is separated from the ectoderm. The nasal pits are larger and more distinct. The sinus terminalis is now complete.

The fragments of the extra-embryonal tissues attached to these embryos are so imperfect that they do not afford any information concerning the extra-embryonal distribution of primordial germ-cells. Primordial germ-cells are present in the heads of both embryos in the mesenchyme or small blood-vessels close to the fore-brain. These primordial germ-cells are frequently pressed against the outer surfaces of the fore-brain and in contact with it. They do not show any clear signs of degeneration. There are eighteen primordial germ-cells in the head of M 51, but only six in that of M 81.

One primordial germ-cell is present in the liver in each embryo. No others occur in M 51, as the slide containing the sections of the germinal ridge region has disappeared. A considerable number of primordial germ-cells are present in the germinal ridges of M 81 and in their vicinity, either in the aorta and the smaller vessels arising from it or in the mesoderm bounding the mid-gut groove. This distribution suggests that the

majority of the primordial germ-cells have reached the germinal ridges or their immediate vicinity, while some having got carried into the smaller vessels of the head in the blood-stream have become wedged there and are permanently astray.

Stage N.

(Four embryos, N 96, N 14 a, N (Howes), and N 17 a.
Fig. 3, Pl. 16, Fig. 8, Pl. 17.)

The curvative of the body has increased somewhat. The distal extremity of the fore-limb is flattened. Four gill-pouches open to the exterior. The lung buds and the primordium of the dorsal pancreas are present. The Wolffian ducts open into the cloaca. There is no neurenteric canal.

No extra-embryonal primordial germ-cells are present in the yolk-sac splanchnopleure in the one embryo in which a considerable part of the yolk-sac wall is preserved. The only extra-embryonal primordial germ-cells are situated in the wall of the allantois. These facts, coupled with the distribution of the primordial germ-cells within the embryos, suggest that all the primordial germ-cells have reached the neighbourhood of the germinal ridges or have got permanently lost on the way. Aberrant primordial germ-cells are found in the head of all the embryos, and occasional ones are found in various other localities. Most of those in the head are in the region of the fore-brain and especially between its lateral wall and the optic vesicles. No primordial germ-cells are found in the heart or blood-vessels of any of the embryos with the exception of a few in the head region which are in capillaries. It is therefore quite certain that migration by way of the blood-stream has completely stopped at this stage. Many primordial germ-cells are present in the germinal ridges themselves (fig. 3, Pl. 16, fig. 8, Pl. 17), some in the deeper parts of the ridges, and some in the peritoneal epithelium covering them. The latter, on account of their size, tend to project markedly from the surface of the germinal ridge into the coelom. Many of the primordial germ-cells in the germinal ridges exhibit reduction of their content of characteristic yolk-granules, a few having lost these granules altogether. Primordial

germ-cells are also numerous in the mesoderm in the immediate vicinity of the germinal ridges, above them but beneath the aorta, and between them in the region of the insertion of the mesentery. Others are present in the mesentery itself, chiefly in the region of the posterior extremities of the germinal ridges and behind them; some are found even in the mesentery of the hind-gut, and a few in the splanchnic mesoderm forming the walls of the mid-gut groove or in that surrounding the hind-gut. This distribution suggests that the last of the migrating primordial germ-cells are moving through the mesoderm, by their own amoeboid activity, up the mesentery and into the posterior portions of the germinal ridges.

The detailed distribution of the primordial germ-cells in each of the embryos, on which this summary is based, is as follows:

N 96. The embryo, without any of the yolk-sac wall attached, is cut longitudinally. Nine primordial germ-cells are present in the head, of which six are in the fore-brain region, either close to the optic vesicles or in front of them, and three in the mid-brain region. Most of these are embedded in the mesoderm, but one is partly in a capillary, one is surrounded by red-blood corpuscles, and two are close to capillaries. Approximately sixty-six primordial germ-cells are present in the germinal ridges or their immediate vicinity.

N 14 a. The embryo, which has none of the yolk-sac wall attached, is cut transversely. Thirteen primordial germ-cells are present in the head, eleven of which are in the region of the fore-brain and two in that of the hind-brain. Most of these are embedded in the mesoderm, but some are in capillaries. Many are present in the germinal ridges and in their immediate vicinity, and also in the base of the mesentery of the mid-gut at the level of the germinal ridges and behind them.

N (Howes). The embryo, without any yolk-sac wall attached, is cut transversely. Ten primordial germ-cells are present in the head, of which eight are embedded in the mesoderm in the fore-brain region, one lateral to the mesoderm and one in a capillary beside the hind-brain. This embryo is remarkable for the large number of aberrant primordial germ-cells. One is present in the mesoderm beside a spinal ganglion in the trunk, one in the

mesoderm of the tail immediately below the notochord; one in the mesonephros, one in the fore-limb bud, one in the hind-limb bud, four in the mesentery or splanchnic mesoderm of the fore-gut, and three in the mesoderm of the allantoic wall lateral to the allantoic stalk. Many primordial germ-cells are present in the germinal ridges and in the mesoderm close above and between them. Several are present in the mesentery of the posterior part of the mid-gut and the anterior part of the hind-gut at the level of the posterior extremities of the germinal ridges.

N 17 a. Cut transversely with the clear area of the yolk-sac wall (corresponding more or less closely in extent to the original area pellucida) and the margin of the thickened endoderm beyond this present throughout the length of the embryo on both sides. No extra-embryonal primordial germ-cells are present, although so much of the yolk-sac wall is preserved. Only one primordial germ-cell is found in the head, in the mesoderm between the lateral wall of the fore-brain and the optic vesicle. One is present in the mesoderm of the allantoic stalk, two in the liver. Many primordial germ-cells are present in the germinal ridges and in their immediate vicinity, and at the level of their posterior extremities some are present in the mesentery of the mid-gut and in the splanchnic mesoderm forming the lateral walls of the mid-gut groove.

Stages O to R. (Figs. 4 and 5, Pl. 16.)

The phenomenon of the migration of the primordial germ-cells is virtually complete in stage N, since many of them have reached the germinal ridges and the number of aberrant or extra-regional ones in other parts of the body is greatly reduced in comparison with earlier stages. It is therefore unnecessary to give a detailed description of our observations on later stages. Stages O to R exhibit a further reduction in the number which leads to an ultimate complete disappearance of the extra-regional primordial germ-cells. There can be no doubt that those primordial germ-cells which have strayed into the organs more remote from the germinal ridges degenerate *in situ*. This conclusion is based on the decrease in the number of extra-regional primordial germ-cells observed in successive stages and not on direct

observation of degenerating cells, which are difficult, if not impossible, to identify. Probably most of those primordial germ-cells which are observable in stage N outside, but in the immediate proximity of, the germinal ridges, succeed in entering them. However, in stage R extra-regional primordial germ-cells are few or absent. In this stage the majority of the germ-cells have entirely lost the content of yolk-spherules which is so characteristic of the primordial germ-cells in the earlier stages. Moreover, many of the germ-cells have nuclei in what we believe to be the prophase of the meiotic division.

The fixation is not adequate in this material to justify a detailed description of the nuclear stages, but some of the nuclei exhibit loops of chromatin polarized towards one pole of the nucleus and exhibiting contraction. We believe these to be zygotene or pachytene nuclei in synizesis, since they closely resemble these stages in the adult testis of *Sphenodon*. A primordial germ-cell with its nucleus in this stage is drawn in fig. 5, Pl. 16, with a spermatocyte, in a similar stage, from an adult testis in fig. 6, Pl. 16, for comparison. No contraction was observed in the prophase stages of somatic mitosis observed in the same embryo or in any of the others. It may be concluded therefore that these primordial germ-cells have actually entered upon the prophase of the heterotypic division. A few of them still retain part of their original yolk-content in the form of a few spherules considerably larger than those in the primordial germ-cells of early stages. Some of these yolk-containing germ-cells also exhibit unmistakable prophase stages (figs. 4 and 5, Pl. 16). Since yolk has been entirely absent from the other embryonal cells for a long time, there can be no doubt that these yolk-containing germ-cells are true primordial germ-cells. They provide, therefore, almost conclusive evidence that some, at least, of the primordial germ-cells after reaching the germinal ridges enter on the prophase of the heterotypic division.

DISCUSSION.

The collection of embryos of *Sphenodon*, made by the late Professor Dendy, which have furnished the material of this paper, is remarkably complete from the point of view of the history of

the primordial germ-cells. It has been found possible to trace in them the formation of the primordial germ-cells in the yolk-sac wall at a very early stage of development, their subsequent migration through the tissues or in the blood-stream to the forming germinal ridges, and their ultimate entrance upon the prophase stages of the heterotypic division, characteristic of germ-cells. Moreover, the material is almost unique¹ on account of the ease with which the primordial germ-cells can be identified and distinguished from all other cells of the embryo owing to their large size and characteristic content of yolk-granules.

The primordial germ-cells of *Sphenodon* are formed exclusively in the endoderm of the yolk-sac wall. Their formation must begin at an extremely early stage of development, since some are already differentiated (fig. 1, Pl. 16) and others are in process of formation in the yolk-sac wall of stage C, the earliest at our disposal. The embryo of this stage has no clearly differentiated medullary plate. Primordial germ-cells continue to differentiate in stages D and E, and may possibly do so even later in the parts of the yolk-sac wall more remote from the embryo. The primordial germ-cells form in the deeper layers of the yolk-sac endoderm of the area opaca (fig. 2, Pl. 16). Few, if any, are formed in the endoderm of the area pellucida. They appear to form chiefly at the margin of the area opaca where it joins the area pellucida, and, though forming all round the embryonal shield, do so in greatest numbers in front of and lateral to the developing head-fold (Text-figs. 1 and 3). The chief area of differentiation is therefore in the form of a crescent surrounding the area pellucida in front of the embryonal shield. The yolk-sac endoderm in which they form consists of a loose network of stellate cells in the superficial layers of the yolk. Yolk-spheres are scattered plentifully between the cells. The endoderm cells themselves are of various sizes, are stellate in shape, and either do not contain yolk or else contain in their cytoplasm yolk-granules of various sizes. The forming primordial germ-cells are much larger than the endoderm cells and are also stellate in shape, often with long cytoplasmic processes in contact with

¹ We limit our remarks to the Sauropsida. A comparative account is to be found in a recent publication by one of us (F. W. R. B.) (8).

those of neighbouring cells (fig. 1, Pl. 16). They are also characterized by their rich content of yolk-spherules which are small and, unlike those in the endoderm cells, are approximately equal in size. These distinctive yolk-granules are numerous and scattered throughout the cytoplasm. Moreover, they are a constant feature of the primordial germ-cells from the time of their formation until after they have entered the germinal ridges. The primordial germ-cells, when completely differentiated, lose their stellate shape and become roughly spherical or ovate in form (fig. 3, Pl. 16).

The primordial germ-cells, almost as soon as they are formed, begin to move through the yolk-sac endoderm, apparently by their own amoeboid activity. They invade the endoderm of the area pellucida and are found in it in considerable numbers in stages D and E, and are found in the yolk-sac endoderm of the bilaminar blastoderm above the head in the latter stage, as well as in F and H. They are still more numerous in these stages around the margin of the area opaca where it joins the area pellucida. Apparently these primordial germ-cells which are in the yolk-sac endoderm of the bilaminar blastoderm (which is in continuity with that of the proamnion), in front of, above and lateral to the head, migrate outwards and backwards into the yolk-sac splanchnopleure, at the level of the junction of the bilaminar blastoderm with the proamnion, and so come to lie on each side of the trunk of the embryo.

A remarkable strand-like cluster of primordial germ-cells is present in stage E lateral to the embryo in the region of the primitive knot (streak). This aggregation of primordial germ-cells is situated in the yolk-sac endoderm on each side at the point where the area pellucida merges with the area opaca and close to the lateral margin of the sub-germinal cavity (Text-fig. 4). This cluster is, however, transitory and soon breaks up. The constituent primordial germ-cells become scattered in the extra-embryonal splanchnopleure lateral to the embryo, considerably augmenting the number found there.

Primordial germ-cells occur in the endoderm only where the blastoderm is bilaminar, but are present in the mesoderm as well where it is trilaminar. They occur in the splanchnopleure, but

not in the somatopleure, where the extra-embryonal mesoderm is split by the coelom. They tend especially to accumulate in the blood-islands and in the sinus terminalis as soon as these are formed and even while they are still solid. As soon as the embryonic heart begins to beat, which was observed by Dendy in the living embryo of stage J, and the circulation begins, they get carried in the blood into the vitelline veins.

The first stage in which primordial germ-cells are observed within the embryo is in stage K. Evidently they enter the embryo by either of two ways:

First, those that wander into the blood-islands, sinus terminalis, or the vitelline vessels get carried passively into the embryo in the blood-stream (Text-fig. 8). They enter the embryo by way of the vitelline veins, pass through the heart and aortic arches, and reach the dorsal aorta (Text-fig. 9). Some get carried into the cardinal veins and travel in them into the head until they stick in the lumen of a capillary. Others get carried in smaller numbers to most of the other organs of the body. These primordial germ-cells, which have lost their way, finally penetrate the walls of the capillaries in which they are jammed and remain in the adjacent tissue until finally they degenerate. The majority of the primordial germ-cells are carried in the blood-stream down the dorsal aorta towards the vitelline and mesonephric arteries. This is their opportunity, since these arteries come off from the aorta at the level of the developing germinal ridges. The primordial germ-cells, when they reach this neighbourhood, penetrate the walls of the vessels and enter the surrounding mesoderm through which they migrate into the germinal ridges on each side. Doubtless, some of the primordial germ-cells, failing to get out of the vessels, get carried out of the embryo through the vitelline arteries. Presumably they enter the embryo again in the venous blood-stream.

Second, some of the primordial germ-cells appear not to enter the blood-stream but to migrate through the extra-embryonal endoderm and mesoderm into the embryo. This active migration ultimately brings them into the splanchnic mesoderm bounding the mid-gut groove. From there they move up into the base of the mesentery of the mid-gut (fig. 7, Pl. 17), and then laterally

into the forming germinal ridges. Thus the primordial germ-cells reach the tissues above the mesentery by two ways (Text-fig. 11), and move from there into the germinal ridges at the time when they are scarcely formed. The future site of the germinal ridges at this stage is clearly delimited by the base of the mesentery on one side and by the mesonephros on the other. The developing germinal ridge is covered by a single layered coelomic epithelium which has not begun to proliferate at the time when the primordial germ-cells enter it (fig. 8, Pl. 17).

The primordial germ-cells occur first in the vitelline veins outside the embryo in stage J. They are found in the embryo in stage K (Text-fig. 9). The majority are in the embryo in L and those that are extra-embryonal are close to the embryo. All the primordial germ-cells have entered the embryo in N, and except for those that have gone permanently astray they have all reached the germinal ridges or their immediate vicinity and are no longer in the circulation. The primordial germ-cells may thus be considered to have completed their migration at this time. Subsequently those that have failed to reach the germinal ridges degenerate, and by the time stage R is reached no primordial germ-cells are found outside the germinal ridges.

It is outside the scope of this paper to deal with the controversial subject of whether secondary germ-cells arise from the germinal epithelium, and with the correlated problem of whether the primordial germ-cells degenerate entirely or persist to give rise to some or all of the definitive germ-cells. The material available does not permit us to express any opinion on these subjects. It has been shown that the primordial germ-cells after entering the germinal ridges lose their characteristic content of yolk-granules. The germ-cells in stage R apparently exhibit the prophase stages of the heterotypic division. The majority of them are devoid of yolk and therefore cannot be positively identified as primordial. Some exhibiting these prophase stages do contain yolk-granules and therefore can be definitely identified as primordial germ-cells. It is therefore concluded that some, at least, of the primordial germ-cells enter upon meiosis whatever their ultimate fate may be.

The results recorded in this paper differ in several important

respects from those obtained by other workers on the primordial germ-cells of reptiles. The chief papers previously published on reptiles are those of Allen (1, 2, 3) and Dustin (5) on the turtle, *Chrysemys marginata*.

Allen (1) states that the primordial germ-cells of *Chrysemys* are first observed in the endoderm (embryo 1.7 mm. long) at the edge of the area pellucida, in a zone on each side of the embryo extending from a point opposite the anterior portion of the pronephros to a point behind the embryo. The primordial germ-cells are clearly distinguished from the other cells by their large size, spherical form, and the presence in them of numerous yolk-spherules of varying size. They migrate from their site of origin through the endoderm to the apex of the mid-gut groove. There they move into the mesoderm and migrate up the mesentery to the primordia of the germinal ridges on each side. Allen (2) finds that about 50 per cent. of the primordial germ-cells complete their migration and reach the germinal ridge. He observes the persistence for a long time of those primordial germ-cells which fail to reach the germinal ridges.

Dustin (5) confirms all the more important points of Allen's work. The primordial germ-cells are observed in the earliest stage examined, which is an embryo 1.2 mm. total length in which neither the medullary groove nor notochord is formed. The primordial germ-cells, which are scarcely differentiated from the other endoderm cells, are situated on each side of the posterior third of the embryonal area in the endoderm at the limit of the area opaca and the area pellucida. They are clustered together so as to form in transverse section a spindle-shaped thickening in the endoderm. Dustin is unable to find any behind the embryo, as Allen does. The primordial germ-cells migrate from this site towards the middle line, some entering the mesoderm, but the majority moving through the endoderm. Those from both sides assemble in the endoderm at the apex of the mid-gut groove and move into the mesoderm and up the mesentery as it elongates. They become grouped in the base of the mesentery in this way and remain there for a short time before moving out on each side into the forming germinal ridges. Many of the primordial germ-cells fail to complete the migration and become

lost on the way. Dustin lays great stress upon the grouping of the primordial germ-cells at three stages; first in the endoderm on each side, then in the mesoderm at the base of the mesentery in the middle line, and finally in the germinal ridges on each side. He gives to these three successive localizations the names of primary paired glands ('glandes paires primaires'), median unpaired gland ('glande impaire médiane'), and secondary or definite paired glands ('glandes paires secondaires ou définitives'), and takes Allen to task for not recognizing these three significant stages.

Allen (3), replying to Dustin, reaffirms his contention that primordial germ-cells occur behind the embryo in early stages, and that they are thus distributed in a horseshoe around the posterior end of the embryo. He also doubts the existence of the primary paired, unpaired, and definitive paired glands, and considers that Dustin applies these names to what are merely points on the migration path of the primordial germ-cell; a conclusion with which we are inclined to agree in the light of our observations on *Sphenodon*.

Our results on *Sphenodon* agree with those of Allen and Dustin on *Chrysemys* concerning the extremely early formation of the primordial germ-cells in the yolk-sac endoderm and on their distinctive cytological characters. Indeed, the primordial germ-cells of *Sphenodon* are larger in proportion to the other cells and contain even more yolk-spherules than those of *Chrysemys*, judging by the figures. The major points of difference between our results and those of Allen and Dustin are:

(1) In *Sphenodon* the primordial germ-cells are formed all round the embryo at the margin of the area opaca where it joins the area pellucida, but chiefly in a horseshoe round the anterior end of the embryo. In *Chrysemys* the primordial germ-cells are formed laterally to the posterior third of the embryo and, according to Allen, behind it also, but not around the anterior end at all.

(2) The migration in *Sphenodon* is partly *via* the splanchnic endoderm and mesoderm to the mesentery of the mid-gut, and partly *via* the vitelline veins and the circulation to the mesoderm surrounding the dorsal aorta in the region of the

mesonephros. The migration in *Chrysemys* is *via* the endoderm to the mid-gut groove and thence through the mesoderm up the mesentery to the region of the germinal ridges, but not *via* the circulation.

Sphenodon seems to resemble birds more closely than *Chrysemys* in these respects. Swift (7) describes, and Reagan (6) confirms experimentally, that the primordial germ-cells in the chick arise in the endoderm of the 'germ-wall' at the margin of the area pellucida anterior and antero-lateral to the embryo. They occupy the space between the endoderm and ectoderm of the bilaminar omphalopleure. Later they wander into the mesoderm as it extends and enter the blood-islands and vessels as they form. They get carried in the blood-stream to all parts of the embryo, but collect in the vessels of the splanchnic mesoderm in twenty to twenty-two somite embryos. Subsequently they disappear from the blood-stream and are found in the splanchnic mesoderm near the angle of the coelom in twenty-three to twenty-five somite embryos, and from here pass into the germinal ridges as they form.

Thus *Sphenodon* resembles the fowl in that the primordial germ-cells arise in a crescentic area around the anterior end of the embryo, and resembles *Chrysemys* in that they arise also, though in small numbers, around the posterior end. It further resembles the fowl in that some of the primordial germ-cells migrate passively in the blood-stream, while it resembles *Chrysemys* in that other primordial germ-cells migrate actively through the splanchnopleure to the germinal ridges. *Sphenodon* in these respects is exactly intermediate between *Chrysemys* and *Gallus*.

SUMMARY.

1. The primordial germ-cells of *Sphenodon* originate in the yolk-sac endoderm of the area opaca all round the embryo, but chiefly in a crescentic area in front of it.
2. They differentiate first at a very early stage of development before the differentiation of the medullary plate.
3. The primordial germ-cells are characterized by their very large size, in comparison with all the other embryonal cells, and

by their content of small yolk-spherules which are sub-equal in size.

4. The primordial germ-cells migrate through the yolk-sac endoderm and mesoderm, apparently by their own power of amoeboid movement. Many of them enter the blood-islands and the sinus terminalis.

5. The primordial germ-cells enter the embryo either (1) passively in the venous blood-stream, or (2) actively by migration through the extra-embryonal endoderm and splanchnic mesoderm into the lateral walls and the mesentery of the mid-gut groove.

6. The primordial germ-cells in the circulation reach the neighbourhood of the germinal ridges in the dorsal aorta or its branches. They then penetrate the walls of the vessels and migrate through the intervening tissues, together with those that have reached the base of the mid-gut mesentery by way of the splanchnic mesoderm and the mid-gut wall, to the germinal ridges.

7. Many primordial germ-cells get lost during their migration. This is especially true of those travelling in the blood-stream. Such aberrant primordial germ-cells are found occasionally in almost any part of the embryo, but occur most often in the head, especially in the region of the fore-brain. They ultimately disappear.

8. The primordial germ-cells enter the forming germinal ridges before the coelomic epithelium covering them has begun to proliferate.

9. The primordial germ-cells, having reached the germinal ridges, lose their characteristic yolk-content and enter on the prophase of the heterotypic division.

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ADDENDUM.

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EXPLANATION OF PLATES 16 AND 17.

The illustrations are drawn with the aid of a camera lucida.

PLATE 16.

Fig. 1.—Primordial germ-cell in the yolk-sac endoderm of stage C, showing the typical content of yolk-spherules and the appearance of the neighbouring endoderm cells and yolk-spheres. *E*, yolk-sac endoderm cell; *PGC*, primordial germ-cell; *Y*, yolk-sphere.

Fig. 2.—Three primordial germ-cells full of small yolk-spherules, in the yolk-sac endoderm of the area in front of the head of the embryo of stage E (embryo 56). The ease with which they can be distinguished by their large size and characteristic yolk-content is clearly shown. *E*, yolk-sac endoderm; *PGC*, primordial germ-cell.

Fig. 3.—Single primordial germ-cell from stage N (embryo of Howes) showing the typical cytological characters. *PGC*, primordial germ-cell.

Fig. 4.—Single primordial germ-cell from stage R (embryo 144). Most of the yolk has been absorbed but some still remains in the form of a single large globule. The nucleus has entered on the prophase of the heterotypic division and is in the lepto-zygotene stage. *PGC*, primordial germ-cell.

Fig. 5.—Single primordial germ-cell from stage R (embryo 144). Several yolk-globules of different sizes can be seen in the cytoplasm. The nucleus has entered on the prophase of the heterotypic division and is in the pachytene stage. It exhibits synizesis, which distinguishes these nuclei from those of somatic cells in the prophase of mitosis.

Fig. 6.—Primary spermatocyte in the testis of an adult *Sphenodon* for comparison with Fig. 5. The nucleus is in the pachytene stage and exhibits synizesis.

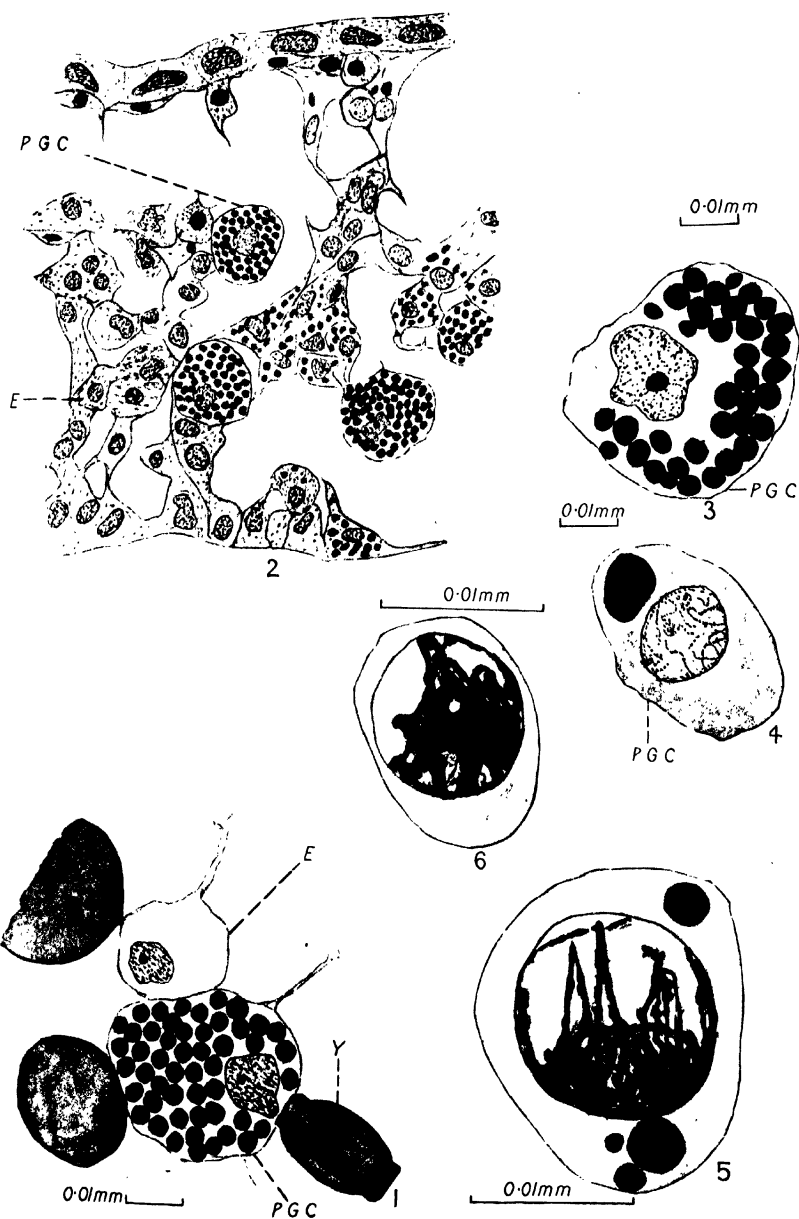
PLATE 17.

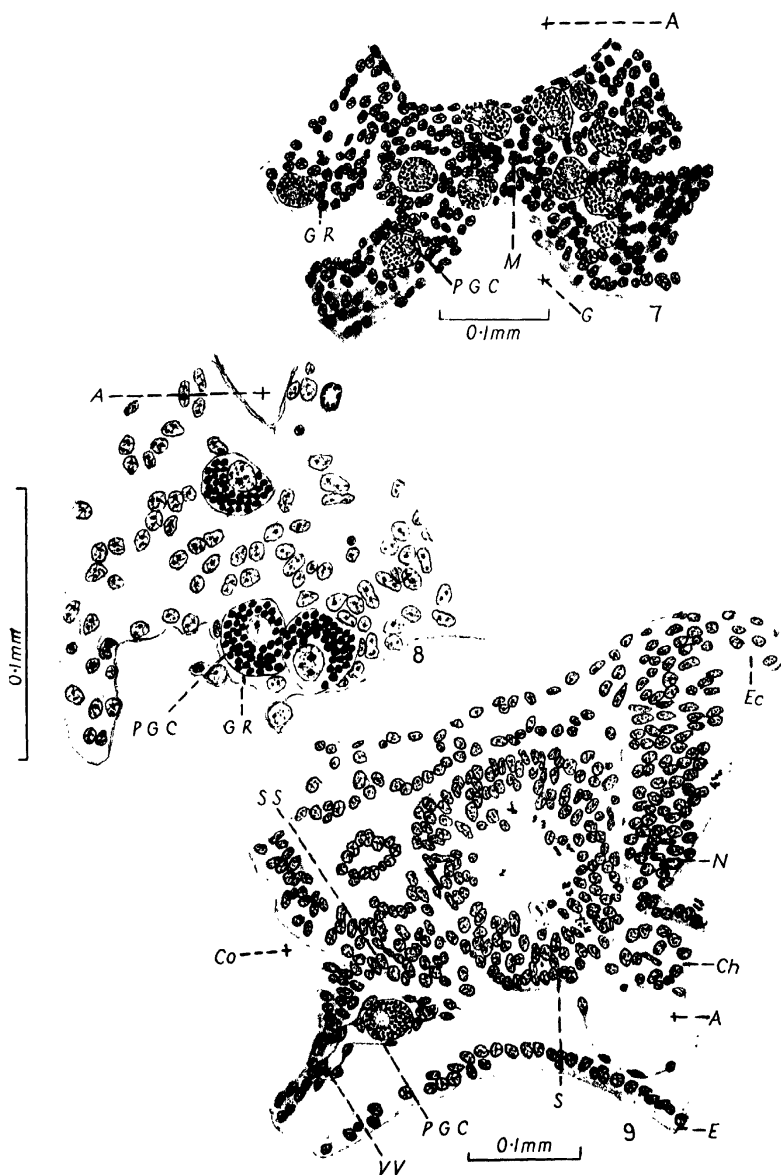
Fig. 7.—Transverse section through the mid-gut groove and mesentery of stage L (embryo 50), showing many primordial germ-cells in the mesoderm of the mesentery and lateral walls of the mid-gut groove. One primordial germ-cell, on the extreme left, is situated in the germinal ridge. *A*, aorta; *G*, mid-gut groove; *GR*, germinal ridge; *M*, mesentery; *PGC*, primordial germ-cell.

Fig. 8.—Transverse section of the germinal ridge and its immediate vicinity in stage N (embryo of Howes). Two primordial germ-cells are situated in the germinal ridge and one in the mesoderm above it. *A*, aorta; *GR*, germinal ridge; *PGC*, primordial germ-cell.

Fig. 9.—Transverse section of part of the embryo of stage K (embryo 39) showing a single primordial germ-cell in a branch of the vitelline vein below the somitic stalk. *A*, dorsal aorta; *Co*, coelom; *Ch*, notochord; *E*, endoderm; *Ec*, ectoderm; *N*, medullary tube; *PGC*, primordial germ-cell; *S*, somite; *SS*, somitic stalk; *VV*, vitelline vein.

0.1mm





The Development of the Alimentary Canal in *Pieris Brassicae* and the Endodermal origin of the Malpighian Tubules of Insects.

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With Plate 18 and 9 Text-figures.

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1. INTRODUCTION.

IN a previous paper I have attempted to give a detailed account of the structure of the alimentary canal in *Vanessa urticae* (Henson, 1931). During the course of that work it became clear that accurate morphological definitions of the various parts of the gut would only be possible after a detailed study of its embryology.

The structure of the larval Malpighian tubules had indicated that, in spite of their position on the hind-gut and their apparent derivation from the proctodaeum, they were not necessarily ectodermal and were indeed more likely to be endodermal derivatives. Their development was thus of considerable significance in relation to general embryological interpretation.

My best thanks are due to Prof. W. Garstang and Prof. F. Balfour Browne for their kindly encouragement and criticism.

2. INTERPRETATION OF THE INTERSTITIAL RINGS.

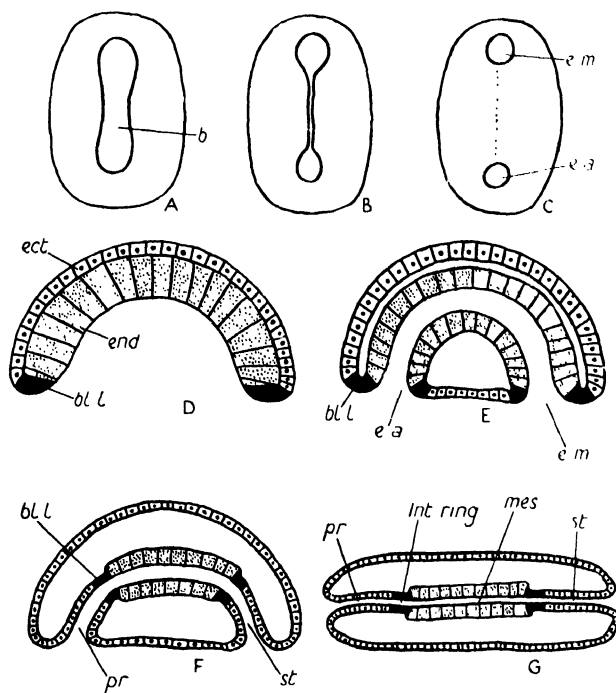
In the previous paper cited above it has been shown that in the larval alimentary canal there are two rings of cells which are persistently embryonic throughout life. One, the anterior interstitial ring, lies between the fore-gut and mid-gut, and the other, the posterior interstitial ring, between the mid-gut and hind-gut. Their presence renders the exact boundary between the mid-gut and the stomodaeal and proctodaeal parts of the alimentary canal rather indefinite (vide Text-fig. 7, and figs. 6 and 9, Pl. 14, loc. cit.). I expressed the opinion that they were the persistent embryonic ends of the stomodaeum and proctodaeum which grew inwards in the embryo by a kind of terminal meristem. Subsequent investigation of embryos has shown that this idea is untenable and some other conception is necessary.

If we attempt to define the interstitial rings we should have to say that they are regions where the ectoderm of the stomodaeum and proctodaeum runs indistinguishably into the endoderm of the mesenteron. This type of definition is applicable to the lips of the blastopore in many animal embryos, and shows that the correct interpretation of these rings may involve the general theory of gastrulation in insects.

Sedgwick (1885) has shown that the blastopore in *Peripatus capensis* divides into two parts by closure of its middle region. The two entrances into the gut thus left are spoken of by him as embryonic mouth and anus. These are subsequently carried inwards by the ingrowing stomodaeum and proctodaeum but are never closed. The relationship of the germ-layers before and after this process and the position of the blastopore lips are indicated in Text-fig. 1. It will be seen at once that the blastopore lips are exactly in the position of the interstitial rings of the insect alimentary canal.

The development of the interstitial rings in the embryo insect should thus furnish an invaluable guide to the exact position of the blastopore lips. In no embryo, however, has it been possible to see any histological differences between those cells which become the interstitial rings and those of the rest of the stomodaeum and proctodaeum. That there is some

essential-difference is only revealed by their failure to differentiate in post-embryonic phases.



TEXT-FIG. 1.

Gastrulation in *Peripatus capensis* illustrating the relationships of the blastopore lips to the interstitial (imaginal) rings of insects. A, B, and C, diagrams of surface views of a *Peripatus* gastrula showing the closure of the middle part of the blastopore. D, a longitudinal section of a gastrula before the closure. E, a longitudinal section after closure. F, a longitudinal section of a *Peripatus* embryo after the formation of the stomodaeum and proctodaeum. G, a generalized longitudinal section of a caterpillar for comparison with F. *b*, blastopore; *bll*, cells of the blastopore lip; *e a*, embryonic anus; *ect*, ectoderm; *e m*, embryonic mouth; *end*, endoderm; *int ring*, interstitial ring; *mes*, mesenteron; *pr*, proctodaeum; *st*, stomodaeum.

The ingrowths from the germ-band usually spoken of as stomodaeum and proctodaeum might not be purely ectodermal in insects. As will be shown later their blind ends develop into

quite definite mid-gut cells. The rim immediately adjacent to the blind end becomes in each case the interstitial ring. I therefore suggest that the so-called proctodaeal and stomodaeal ingrowths in insects are complex structures composed of the proctodaeum and stomodaeum proper, those portions of the blastopore lips corresponding to the embryonic mouth and anus of *Peripatus*, and a portion of true endoderm internally.

3. RELATION OF THE ATTACHMENT OF THE MALPIGHIAN TUBULES TO THE INTERSTITIAL RINGS.

In examining the literature with regard to the development of Malpighian tubules I have found nothing beyond the statement that they arise as outgrowths from the proctodaeum. On this evidence embryologists appear to regard them as ectodermal derivatives. The viewpoint depends entirely on the assumption that the proctodaeal ingrowth is proctodaeum and nothing else. If, however, the blind end of the proctodaeum in insects contains both endoderm and the homologue of blastopore lips the view is open to question. The situation is very similar to that with regard to the mid-gut. Many authors, Mansour (1927), Leuzinger, &c. (1926), have regarded the mesenteron as ectodermal because it arises in association with the ends of the stomodaeum and proctodaeum. For a full discussion of this subject see Eastham's Review (1930).

The organs we know as Malpighian tubules have been found in Amphipod Crustacea, certain Arachnids, and Insects. They have long since been recognized as mesenteron appendages in the Crustacea and Arachnida; it is only in the insects that an ectodermal derivation from the proctodaeum has been claimed for them. This is rather curious in view of the fact that in many insects they are distinctly attached to the mesenteron and not to the hind-gut. In the past it has been customary to deny the homology of the Malpighian tubules in the three groups. I believe they are homologous and endodermal throughout.

As regards the Amphipod Crustacea they have been described in *Melita*, *Corophium*, *Gammarus*, *Orchestia*, *Talitrus*, &c. Baldwin Spencer (1885) has given an account

of their anatomy in *Gammarus* and *Talitrus*. They quite definitely open into the posterior end of the mid-gut. Percyaslawzewa (1888—quoted from Korschelt and Heider) describes them as arising in the embryo as mid-gut diverticula. Baldwin Spencer concluded that they were indisputably endodermal and therefore not homologous with the tubules of insects. I prefer to regard their well-established endodermal nature in this group as an indication of their primarily endodermal nature throughout the Arthropods.

In *Lithobius* there are two Malpighian tubules, one on each side of the body, opening into the hind end of the mid-gut (fig. 1, Pl. 18). The proximal end of the tubule is expanded to form an ampulla. The position is exactly like that described above for the Amphipod Crustacea.

In insects the attachment of the tubules seems to vary. In some groups they open into the mid-gut, in others into the hind-gut. Only a few examples will be given here.

Davis (1927) writing of *Stenopelmatus* (Orthoptera) describes six ureters (i.e. proximal ampullae receiving the tubules proper) lined with the kind of epithelium typical of the mid-intestine, and opening into the posterior end of this organ. The morphology seems to be quite comparable with that of *Lithobius* and the Crustacea except for the increased number of tubules.

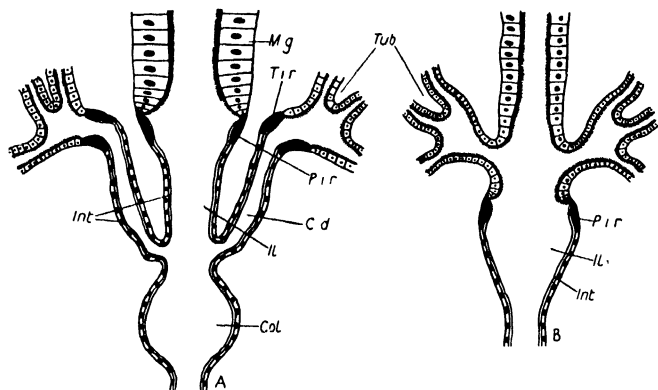
I have personally verified the fact that even in a specialized form like *Calliphora* (Diptera) the tubules have no association with the hind-gut (vide also Pérez, 1910).

In the Lepidoptera the morphology of the attachment of the tubules is extremely interesting and illuminating. In *Hepialus* the tubules open into the posterior end of the mid-gut (fig. 2, Pl. 18); apparently also in *Pleretes* (Bordas, 1911). In *Pieris* they open into the hind-gut. Text-fig. 2 shows diagrammatically the relationships of the attachments of the tubules to the gut in *Hepialus* (B) and *Pieris* (A).

It will be seen that in *Hepialus* they are outgrowths from the mid-gut. They are strictly comparable in position with those of the Amphipod Crustacea, Myriapoda, and Orthoptera. Their proven endodermal derivation in the Amphipoda is thus

a strong indication of their fundamentally endodermal nature in *Hepialus*.

Text-fig. 2, A shows the same regions in *Pieris brassicae*. Here there is a long common duct lined with a chitinous intima and opening into the colon. Where this joins the tubules proper is a ring of embryonic cells exactly like the posterior interstitial ring of the gut. This ring has been observed in many



TEXT-FIG. 2.

Diagrammatic representations of the attachments of the Malpighian tubules in *Pieris* (A) and *Hepialus* (B). *Cd*, proctodaeal part of the common duct in *Pieris*; *Col*, colon; *Il*, ileum; *Int*, chitinous intima covering all parts of the true proctodaeum; *Mg*, mid-gut; *Pir*, posterior interstitial ring; *Tir*, interstitial ring of the Malpighian tubules; *Tub*, endodermal part of the Malpighian tubules.

Lepidoptera and is usually referred to as the imaginal ring of the tubules (Ito, 1921). The chitinous intima does not extend beyond this ring. The tubules proper can scarcely be endodermal in *Hepialus* and ectodermal in *Pieris*, and the fact that the posterior interstitial ring of the gut marks the end of proctodaeal structures, suggests that so also does the interstitial ring of the tubules. If the view be accepted that the posterior interstitial ring of the gut is the homologue of the lips of the blastopore, we can only explain the presence of the interstitial ring in the tubules as due to their separation from the original blastopore lip during development. This implies that

the common duct in *Pieris* is a new development and is not represented in *Hepialus*.

The view that the posterior interstitial ring of the alimentary canal is homologous with the lips of the anal half of the blastopore in *Peripatus* thus enables us to correlate the structure of the Malpighian tubules of insects and explain why they open into the mid-gut in some forms and into the hind-gut in others. It also enables us to regard the tubules of Insects, Crustacea, and Arachnids as homologous and indicates that the association of the tubules with the proctodaeum in insect embryos is a secondary one.

Differences of interpretation with regard to the origin of endoderm in insects are notoriously apparent amongst workers in insect embryology (vide Eastham, 1930). Some derive the endoderm from definite anterior and posterior endoderm rudiments; others deny the endodermal nature of the mid-gut because it arises from the blind end of the stomodaeum and proctodaeum. The views advanced in the present paper enable these varying interpretations to be reconciled within a common explanation.

4. DEVELOPMENT OF THE ALIMENTARY CANAL IN *PIERIS*.

(1) *Cleavage and Gastrulation*.—As regards the early development of *Pieris*, Eastham (1927) has already published a full and well-illustrated account. Accordingly only a condensed history of these phases will be given here, coupled with a new mode of interpretation. For verification of the facts of development reference should be made to Eastham's paper (1927).

The unsegmented egg is a large single cell, in the meshes of whose cytoplasm are embedded considerable quantities of yolk. The cleavage nucleus repeatedly divides; some of the daughter nuclei move outwards to the periphery of the egg and form a blastoderm, others remain behind as yolk nuclei. The latter should, I believe, be interpreted as extra-embryonic endoderm.

At the poles of the egg and along its dorsal side the blastoderm becomes very thin and is known as the serosa. The rest of the blastoderm thickens and becomes the germ-band. Double

folds appear at the edge of this germ-band and grow to form a complete amnion over it. In this process the serosa is extended and the germ-band pushed into the yolk.

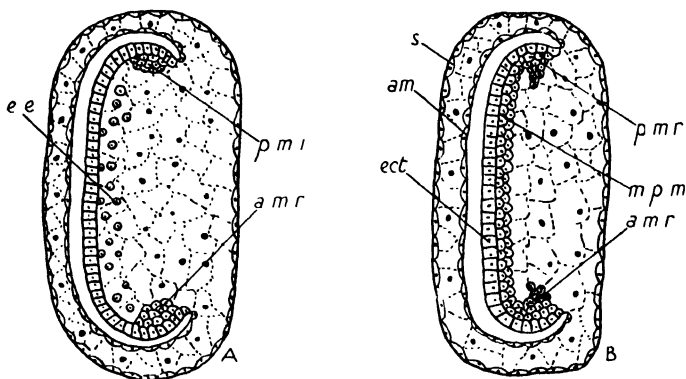
The development up to this point presents some analogies with early mammalian development. After cleavage comes the formation of embryonic membranes which surround a germ-band containing within itself the rudiments of the embryo. Amnion and serosa are readily recognized as extra-embryonic ectoderm.

The anterior end of the germ-band produces by proliferation a heap of cells on its upper side. A wave of cell proliferation then passes down the middle line until it reaches the posterior end when another heap is produced similar to that at the anterior end (Text-fig. 3, A). These two heaps of cells were called by Eastham (1927) anterior and posterior mesenteron rudiments. The cells produced by proliferation along the middle line degenerate; they have been recognized as evanescent endoderm both by Eastham (1927) in *Pieris* and by Mansour (1927) in *Calandra*. As Eastham's later work (1930) shows, however, much of the anterior mesenteron rudiment is mesoderm. My own observations on *Pieris brassicae* show that the same is true of the posterior mesenteron rudiment. With Eastham (1930) I therefore prefer to term them anterior and posterior mesendoderm rudiments. The middle region of the germ-band, i.e. the part which produced the evanescent median endoderm, now sinks inwards and is overgrown by the regions lateral to it (Text-fig. 3, B). This middle plate as it is called now lies on the upper side of the middle of the germ-band as the body mesoderm. The stomodaeum and proctodaeum eventually pass inwards precisely on the site of the anterior and posterior mesendoderm rudiments, thus proving that their position is just that of the embryonic mouth and anus in *Peripatus*.

I suggest that these regions are homologous with the lips of the two halves of the blastopore of *Peripatus*. The site of the production of the anterior mesendoderm rudiment may be called the oral blastoporic area. Similarly the site of the posterior proliferation is an anal blastoporic area. The proliferation along the middle line which produces evanescent endoderm probably represents the closure of the middle part of the blasto-

pore in *Peripatus*. Although this endoderm degenerates in *Pieris* and *Calandra* there is no theoretical reason why it should do so in all insects. Indeed its occasional persistence may explain the results of those authors who derive the mid-gut partly from splanchnic mesoderm (vide Eastham's Review, 1930).

It follows from this interpretation that the blind ends of the stomodaeum and proctodaeum are not ectodermal but are



TEXT-FIG. 3.

Later development of *Pieris rapae*. (A) proliferation from the germ band to produce the anterior and posterior mesendoderm rudiments and the evanescent median endoderm. B, longitudinal section after the sinking inwards of the middle plate to form the body mesoderm. *am*, amnion; *amr*, anterior mesendoderm rudiment; *ect*, ectoderm; *ee*, evanescent median endoderm; *mpm*, middle-plate mesoderm; *pmr*, posterior mesendoderm rudiment; *s*, serosa.

composed of tissue homologous with the lips of the embryonic mouth and anus of *Peripatus* (i.e. the blastopore lips). This renders it impossible to accept the theory (with its anomalies) that the insect mid-gut is an ectodermal derivation because it arises from the blind ends of these two intuckings.

(2) The fore-gut.—The condition of the anterior mesendoderm rudiment at the stage described above is shown in fig. 3, Pl. 18. It will be observed that ectoderm, mesoderm, and endoderm run indistinguishably into one another at this point.

This state of affairs is not unexpected on the site of a proliferating, if virtual, blastopore.

In fig. 4, Pl. 18, is shown the first sign of the intucking of the stomodaeum. This takes place precisely at the position of the anterior mesendoderm rudiment. The oral blastoporic area (still proliferating cells) is thus carried inwards as the blind end of the stomodaeum.

The inward movement is accompanied by a peripheral spreading of the cells of the anterior mesendoderm rudiment and their separation into definite mesoderm and endoderm. The peripheral spreading produces particular mesoderm masses round the base of the stomodaeum. These are arranged into paired pre-oral masses in front, a pair of antennal masses at the sides, and a pair of pre-mandibular masses behind (fig. 4, Pl. 18). The endodermal parts of the original mesendoderm rudiment are now situated entirely above the pre-mandibular mesoderm and around the blind end of the stomodaeum. Its amount is still being increased by proliferation.

In a slightly later stage (fig. 5, Pl. 18) the stomodaeum is seen to have passed still farther inwards and to have carried the endoderm with it. The line of demarcation between endoderm and ectoderm is not precise and never becomes so. The endoderm now has the form of two masses of cells placed ventrolaterally just posterior to the end of the stomodaeum and connected by a strand across the ventral border of its blind end.

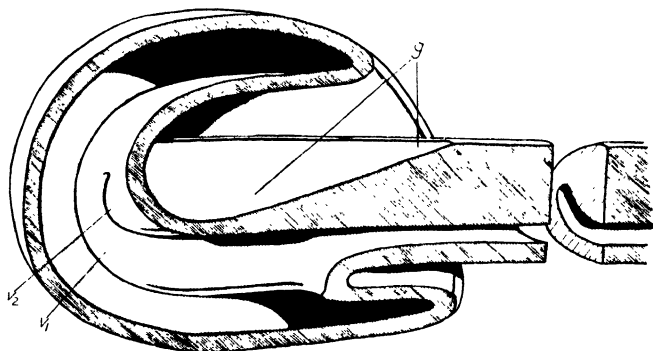
The pre-oral mesoderm has separated into two pairs of cell-groups, labral mesoderm in front and epipharyngeal behind, both placed on the dorsal side of the stomodaeum. Antennal and pre-mandibular mesoderm are much as before (fig. 5, Pl. 18).

Stomodaeum.—Starting as a simple tube the stomodaeum continues to grow inwards and becomes folded in a most complicated fashion (fig. 6, Pl. 18). Its blind end swells out and becomes extremely thin; the thick stalk then pushes into this swollen end to give what is roughly the shape of a mushroom to the whole organ. In Text-fig. 4 is given a stereogram of it at this period. The outer wall is double and folded right back.

The stalk projects into this region in the form of two folds (v_1 and its fellow of the other side) which remain separated along the mid-ventral line. Dorsally there is a much smaller fold v_2 behind which the dorsal wall of the stalk is deeply grooved (g) forming a repository in which lie nerve-masses. (Compare also Text-fig. 6.)

Owing to the great thickness of the dorsal wall of the stalk (Text-fig. 4) its cavity is restricted and U-shaped.

At this stage mesoderm is present between the layers of the



TEXT-FIG. 4.

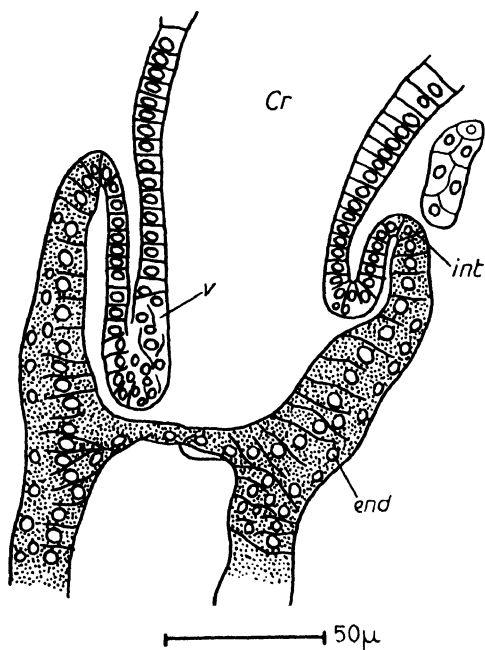
Stereogram of the stomodaeum at the stage shown in fig. 6, Pl. 18.

The figure is supposed to represent an optical section in the sagittal plane. v_1 and v_2 , folds referred to in the text; g , groove. Compare also Text-fig. 6.

folds, but must be left behind during the subsequent elongation of the embryonic folds since there is no mesoderm between the folds of the valve in the larva. In *Pieris* and *Vanessa* this elongation appears to take place by a lengthening of v_1 and its fellow of the other side which carries v_2 backwards and gives the oesophageal valve the form of a split cylinder. Wigglesworth (1930) has described a trifoliate valve in *Cheimabacche*; this is possibly a more primitive condition and due to the individual lengthening of v_1 , its fellow, and v_2 , with retention of the notches between them.

During the course of development the thin outer wall of the blind end of the stomodaeum takes on more and more the

character of endoderm. The process may perhaps be described as a kind of continuous revelation of the endodermal nature of this region. First the ventral border becomes indubitably endodermal (fig. 5, Pl. 18), then the process extends laterally and dorsally (fig. 6, Pl. 18) until finally the whole of this outer wall has the character of endoderm (Text-fig. 5). In this stage



TEXT-FIG. 5.

Median sagittal section showing the late embryonic condition of the stomodaeum. Camera lucida. *cr*, crop; *end*, endoderm; *int*, interstitial ring; *v*, valve.

it remains crossing the gut cavity as a very thin strand which only breaks down just before hatching. We may regard the process as a dwindling of the oral blastoporic area which thereby becomes reduced from a plate covering the end of the stomodaeum to a circle which persists as the anterior interstitial ring. (See my previous paper (1931), fig. 6, Pl. 14.)

Mesoderm and Musculature.—The labral mesoderm situated dorsally on the anterior part of the stomodaeum soon passes forwards into the lengthening labrum and backwards, on either side of the frontal ganglion, to the level of the supra-oesophageal commissure (Text-fig. 6). It differentiates into the whole of the dorsal system of pharyngeal dilator muscles (except possibly the third posterior dorsal). The posterior dorsal dilator muscles lie on either side of the frontal ganglion and in front of the supra-oesophageal commissure.

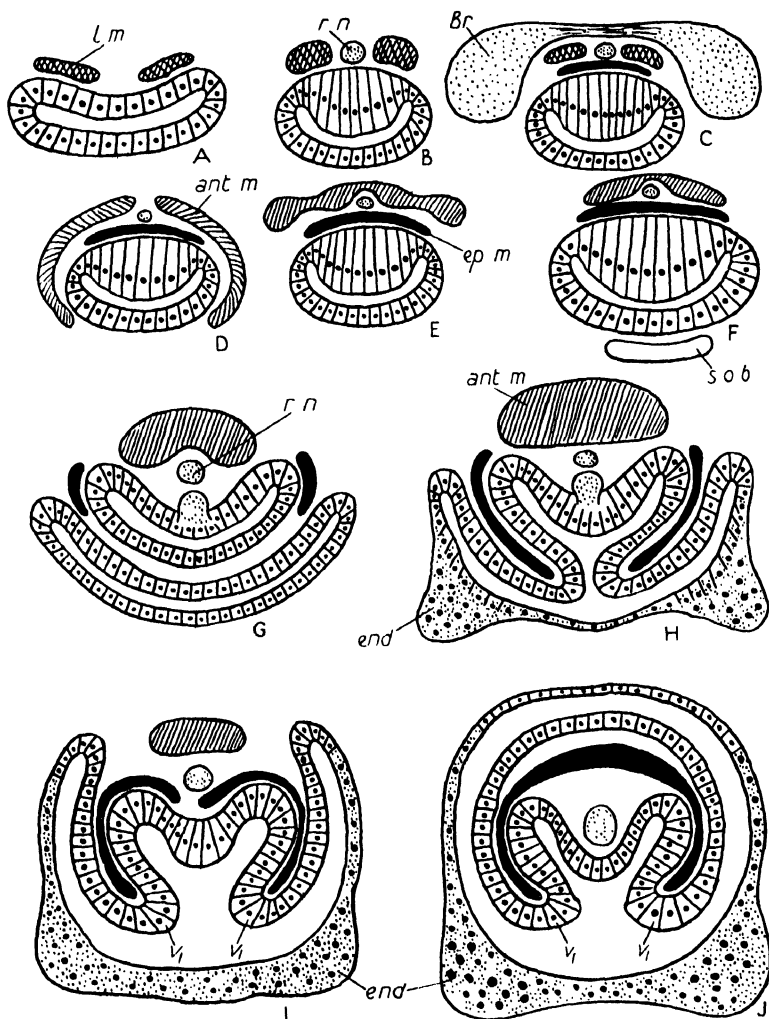
The epipharyngeal mesoderm rapidly surrounds the posterior end of the stomodaeum (Text-fig. 6), and penetrates between the folds (r_1 , r_2 , Text-figs. 4 and 6). It also passes underneath the recurrent nerve and forms a dorsal strip along the main stem of the stomodaeum, as far forwards as the frontal ganglion. Later it passes round on to the lateral and ventral sides in this region also, and differentiates into the transverse and longitudinal muscles of the whole fore-gut. On the pharynx and oesophagus it becomes arranged in six bands (dorsal, dorso-lateral, ventro-lateral, and ventral), but on the crop rudiment it remains a continuous sheath.

The antennal mesoderm is at first lateral to the stomodaeum. Posteriorly it passes upwards on either side of the recurrent nerve and then above it (Text-fig. 6). This dorsally placed antennal mesoderm eventually surrounds the recurrent nerve, passing between it and the epipharyngeal mesoderm. The cephalic aorta is thus formed almost exactly as described by Eastham (1930). A small portion of the anterior more ventrally placed antennal mesoderm passes inwards on the end of the antennal apodeme (i.e. anterior arm of the tentorium) and produces the middle and posterior ventral dilator muscles of the pharynx.

The premandibular mesoderm produces only the sub-oesophageal body.

The anterior ventral pharyngeal dilator muscles seem to be much later in appearing, and are probably derived from labial mesoderm. Most likely they are best regarded as labial muscles and not true pharyngeal dilators.

(3) **The hind-gut.**—The earliest condition which need be considered is that illustrated in fig. 7, Pl. 18. The anal



TEXT-FIG. 6.

Diagrams of serial sections of the stomodaeum to show the distribution of mesoderm. *Br*, brain; *end*, endoderm; *s o b*, sub-oesophageal body or premandibular mesoderm; *l m*, labral mesoderm; *r n*, recurrent nerve; *ant m*, antennal mesoderm; *ep m*, epipharyngeal mesoderm; *r₁*, fold. (Compare also Text-fig. 4, and fig. 6, Pl. 18.)

blastoporic area has produced a few cells by proliferation and these are not yet separated into mesoderm and endoderm.

Before very much proliferation has occurred the proctodaeum begins to pass inwards; separation of the mesoderm then follows. Fig. 8, Pl. 18, shows this stage and strongly suggests that the eleventh abdominal segment is left entirely devoid of mesoderm. We are thus led to conclude that the mesoderm surrounding the proctodaeum really belongs to this eleventh segment.

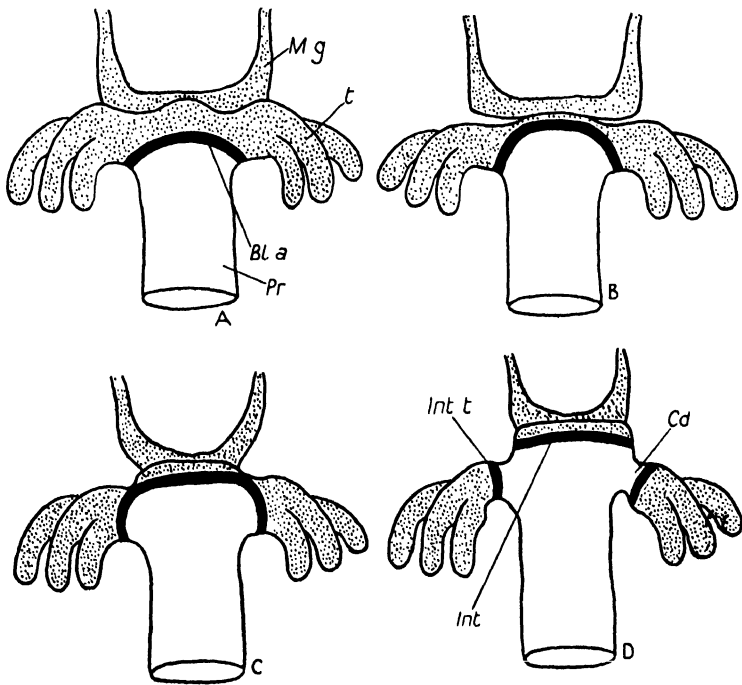
Concurrently with its inception the proctodaeum produces on its blind end a pair of lateral bulges from each of which grow out three Malpighian tubules. These may thus be said to arise as a pair of tri-digitate lobes. The three tubule rudiments lie dorso-laterally, laterally, and ventro-laterally on each side (Text-fig. 7). They pass backwards immediately outside the proctodaeal mesoderm to become closely applied to the ectoderm of abdominal segment eleven (fig. 10, Pl. 18).

The endoderm cells rapidly proliferate and form two compact masses, one on either side in the ninth abdominal segment a little below and behind the blind end of the proctodaeum. These masses may be seen in fig. 9, Pl. 18. In front they are confluent with the lateral bulges bearing the Malpighian tubules and are connected by a strand across the ventral border of the middle region of the proctodaeum (fig. 8, Pl. 18, Text-fig. 8, A). Later they separate from the lateral bulges but always remain attached to the central part (fig. 10, Pl. 18).

To explain the structure of the Malpighian tubules in the larva it has been postulated that they are really endodermal and carry away with them in their subsequent development a small portion of the blastopore lip. It is impossible to point to particular cells in the embryo and say they mark the blastopore lip. In Text-fig. 7 its position is indicated on purely theoretical grounds which the reader may or may not accept. Its greatest justification is the fact that it enables us to make a consistent comparison between the tubules of *Hepialus* which open into the mid-gut and those of *Pieris* which open into the hind-gut.

Text-fig. 7, A, shows an early stage in which the end of the

proctodaeum and the Malpighian tubules are regarded as endodermal and the boundary of the anal blastoporic area is shown in black. We may call this the *Hepialus* phase of development because of its similarity to that form (cf. Text-fig. 2, B). In the next phase (Text-fig. 7, B) growth of the ventral and dorsal



TEXT-FIG. 7.

Theoretical reconstruction of the position and development of the boundary of the anal blastoporic area. *Bl a*, boundary of anal blastoporic area (black); *Cd*, proctodaeal part of the common duct of the tubules which later increases in length; *Int*, interstitial ring of the gut (main part of the blastopore boundary); *Int t*, interstitial or imaginal ring of the Malpighian tubules (i.e. cut-off part of blastopore boundary); *Mg*, mid-gut cells recognizable from the first; *Pr*, proctodaeum.

sides of the proctodaeum has carried the boundary of the blastoporic area forwards above and below, but has left it looping back under the stalks of the tubules at the sides. The reality

of this process may be indicated by reference to fig. 9, Pl. 18, where it has carried the mesoderm forwards as a dorsal and ventral tongue between the tubule attachments. By a continuance of this forward growth, a portion of the boundary of the blastoporic area is left behind on the tubule stalks (Text-fig. 7, C, D), where it later reveals itself as the interstitial ring of the tubules. The main part of the boundary goes on to become the posterior interstitial ring of the gut.

Regions of hind-gut.—The relationships of the proctodaeum to the posterior end of the body in the stage shown in fig. 8, Pl. 18, are better revealed in Text-fig. 8, A, which is a diagram of a coronal section through the proctodaeum. This phase is rapidly followed by one in which the eleventh abdominal segment becomes intucked to form the rudiment of the anterior rectum (Text-fig. 8, B; fig. 10, Pl. 18). The blind ends of the Malpighian tubules come into very close association with this intucked segment and eventually develop complicated anatomical relations with it.

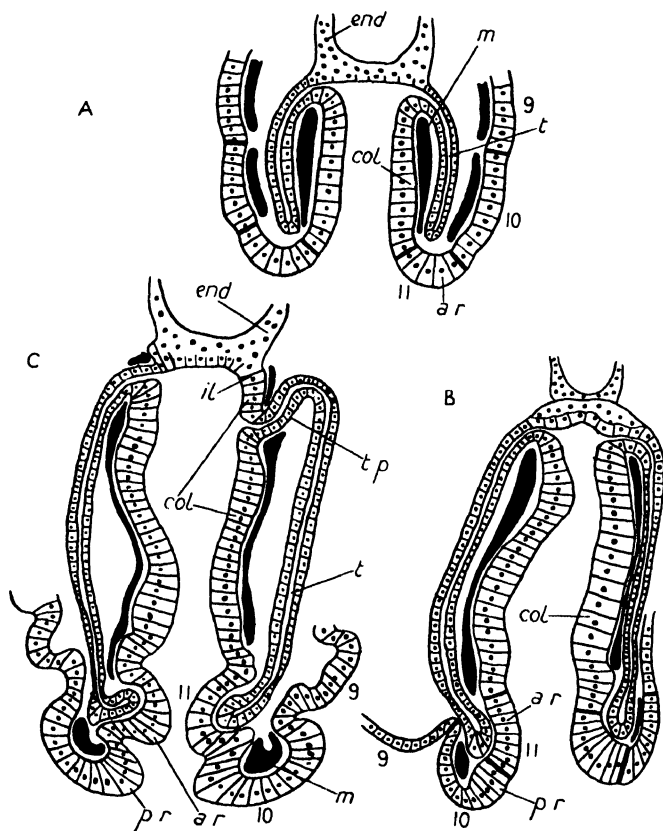
Meanwhile the proctodaeum proper has increased in length as already described. Although the attachment of the tubules would appear at first sight to divide it into colon and ileum, the mesoderm attachments clearly show that this is not so. The mesoderm naturally divides the proctodaeum into ileum and colon (Text-fig. 9), but the tubule attachments are concerned entirely with the colon.

During the lengthening of the proctodaeum the posterior half of the tenth abdominal segment also becomes intucked and forms the rudiment of the posterior rectum (Text-fig. 8, C, and Text-fig. 9).

The mesoderm of the eleventh abdominal segment is, as we have seen, early passed on to the proctodaeum; thus it comes about that the anterior rectum is always devoid of muscles or other mesoderm elements.

The mesoderm continuously grows forwards as the proctodaeum lengthens, and slides like a slip ring over much of the common ducts of the tubules. From the posterior end of the true proctodaeum to a point somewhat in front of the opening of the tubules into the gut, the mesoderm arranges itself in six

longitudinal bands (dorsal, dorso-lateral, ventro-lateral, and ventral). On the anterior end the mesoderm arranges itself in



TEXT-FIG. 8.

Diagrams to illustrate the development of the hind-gut. 9, 10, 11, abdominal segments; *ar*, anterior rectum; *col*, colon; *il*, ileum; *m*, mesoderm; *pr*, posterior rectum; *t*, tubule; *t p*, proctodaeal common duct of tubules; *end*, endodermal mid-gut.

a continuous sheath and not in bands. The anterior region is ileum, the posterior colon (Text-fig. 9, E and C).

The common ducts of the tubules run underneath the ventro-lateral muscle-bands of the anterior third of the colon and have

no muscles of their own. Where they become free from the gut they take this ventro-lateral band with them and thus produce a short piece of the colon with only four muscle-bands (Text-fig. 9, D).

The anterior and posterior sphincter regions are really only the anterior and posterior ends of the colon especially heavily provided with muscles.

In the final stage the blind end of the proctodaeum reveals itself as endodermal (Text-fig. 9, A) by developing an endoderm lamella across the gut cavity just as in the case of the stomodaeum.

Mid-gut.—Little need be said with regard to this since, to Eastham's account, I have only to add that the blind ends of both stomodaeum and proctodaeum are also endodermal, although transitional and destroyed just before hatching.

5. SUMMARY.

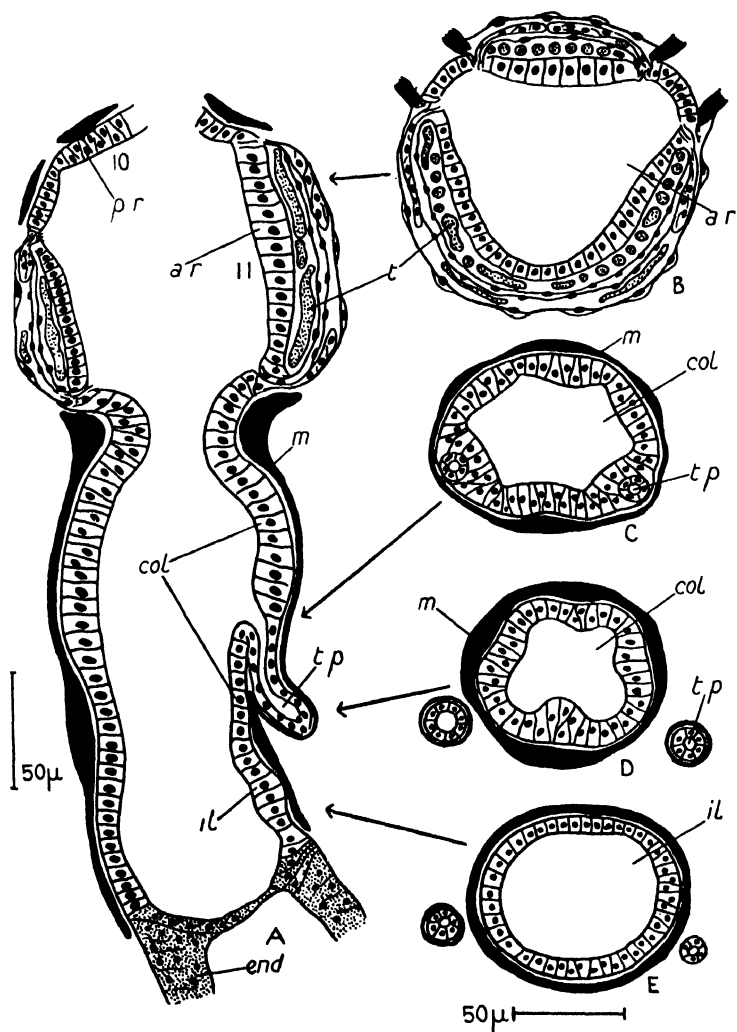
1. The interstitial (imaginal) rings of the insect gut are interpreted as homologous with the lips of the embryonic mouth and anus of *Peripatus* (i.e. the blastopore lips).

2. The Malpighian tubules of Amphipod Crustacea, *Lithobius*, *Stenopelmatus* (Orthoptera), *Hepialus* (Lepidoptera), *Calliphora* (Diptera) are all appendages of the posterior end of the mid-gut and endodermal.

3. The Malpighian tubules of *Pieris*, although hind-gut appendages must be homologous with those of *Hepialus*. They are composed of three regions, (1) the functional parts of endodermal derivation, (2) the interstitial or imaginal ring which is probably derived from the posterior interstitial ring of the gut, (3) the common duct of proctodaeal origin.

4. The germ-band of the Lepidopterous embryo has a closed blastopore or primitive streak composed of two circular areas, anal and oral, connected by a median strand. The anal and oral blastoporic areas produce the anterior and posterior mesoderm rudiments.

5. The development of the stomodaeum and proctodaeum shows that the following characteristics may be ascribed to the various parts of the gut.



TEXT-FIG. 9.

Longitudinal (coronal) and transverse sections of the hind-gut. Arrows point to the regions from which the transverse sections are taken. B, anterior rectum and the membranes covering the terminal parts of the tubules. C, Colon. D, Anterior end of colon with four muscle bands. E, ileum. References as in Text-fig. 8.

Pharynx.—The oral or 'proximal' end of the stomodaeum. Its dorsal dilator muscles are derived from labral mesoderm, its ventral dilators from antennal mesoderm, and its circular and longitudinal muscles from epipharyngeal mesoderm arranged in six bands.

Oesophagus.—The longitudinal and circular muscles are derived from epipharyngeal mesoderm arranged in six bands.

Crop.—Longitudinal and circular muscles derived from epipharyngeal mesoderm not arranged in bands but as a continuous encircling sheath.

Oesophageal Valve.—Primarily trifoliate and formed by outgrowth of three embryonic folds; devoid of mesoderm.

Anterior Interstitial Ring.—The persistent part of the oral blastoporic area and homologous with the lips of the embryonic mouth of *Peripatus*.

Mid-gut.—Endodermal, and formed from those parts of the blastoporic areas internal to the blastopore lips.

Posterior Interstitial Ring.—The persistent part of the anal blastoporic area and homologous with the lips of the embryonic anus of *Peripatus*.

Ileum.—The anterior end of the proctodaeum where the mesoderm forms a complete sheath of muscles not arranged in six bands.

Colon.—The mesoderm is arranged in six bands. The sphincter regions are really the anterior and posterior ends of the colon. The common ducts of the Malpighian tubules enter the colon and derive their muscle-sheath from its ventro-lateral bands.

Anterior Rectum.—The intucked eleventh abdominal segment; closely associated with the terminations of the Malpighian tubules. Its mesoderm early passes on to the proctodaeum so that in subsequent stages it is devoid of mesodermal structures.

Posterior Rectum.—The intucked posterior half of the tenth abdominal segment. Musculature derived from the tenth abdominal somites.

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EXPLANATION OF PLATE 18.

LETTERING.

AM, amnion; *AMP*, ampulla of Malpighian tubule; *AMR*, anterior mesendoderm rudiment; *ANTM*, antennal mesoderm; *10th AS*, *11th AS*, abdominal segments 10 and 11; *ECT*, ectoderm; *END*, endoderm; *EPM*, epipharyngeal mesoderm; *FG*, frontal ganglion; *HG*, hind-gut; *INT*, intima; *LM*, labral mesoderm; *LT*, lateral lobe of proctodaeum giving rise to the Malpighian tubules; *MES*, mesoderm; *MG*, mid-gut; *PG*, protocerebral ganglion; *PIR*, posterior interstitial ring; *PM*, premandibular mesoderm; *PMR*, posterior mesendoderm rudiment; *POM*, pre-oral mesoderm; *PR*, proctodaeum; *RN*, recurrent nerve; *SOG*, sub-oesophageal ganglion; *ST*, stomodaeum; *T*, Malpighian tubule;

T G, tritocerebral ganglion; *T T*, terminal part of Malpighian tubule; *Y K*, yolk. All figures drawn with Camera lucida.

Fig. 1.—Longitudinal section through the attachment of the Malpighian tubule in *Lithobius*. (Compiled from four sections in the same series.)

Fig. 2.—Same in *Hepialus humuli* (half-grown larva).

Fig. 3.—Longitudinal sagittal section of the front end of a 24-hour embryo of *Pieris brassicae*. The anterior mesendoderm rudiment is shown as continuous with the ectoderm on the one hand and the body mesoderm on the other.

Fig. 4.—Same in a 30-hour embryo. The stomodaeum is just beginning to appear.

Fig. 5.—Same in a 48-hour embryo.

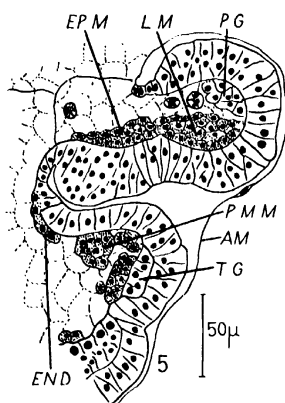
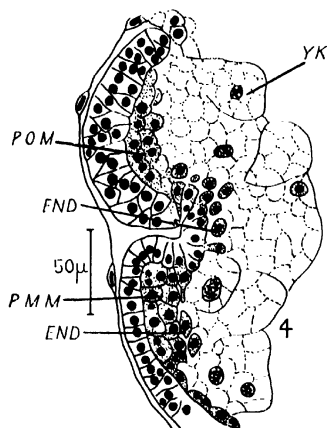
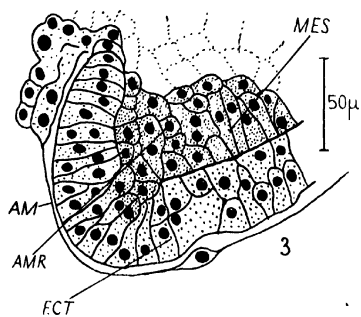
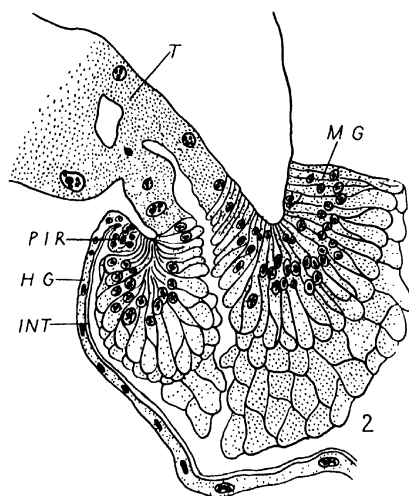
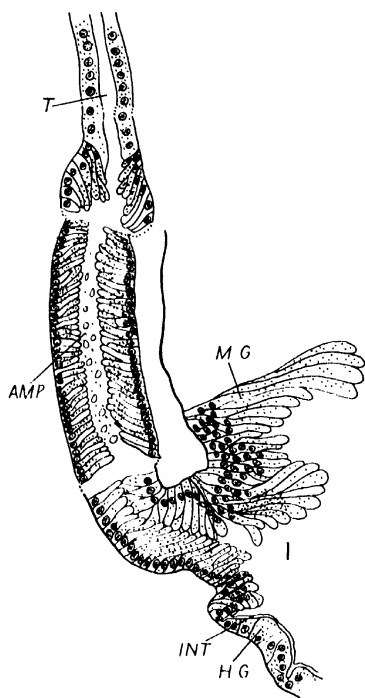
Fig. 6.—Longitudinal sagittal section through the stomodaeum in a 72-hour embryo (*Pieris brassicae*).

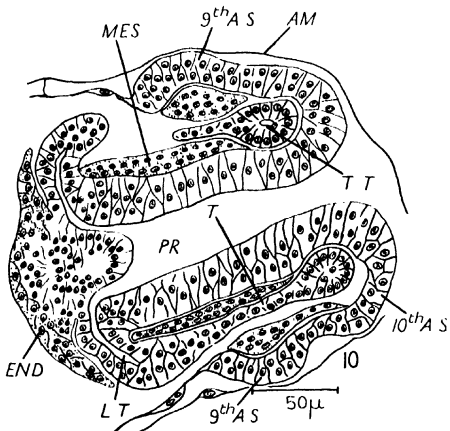
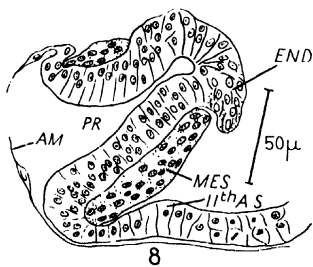
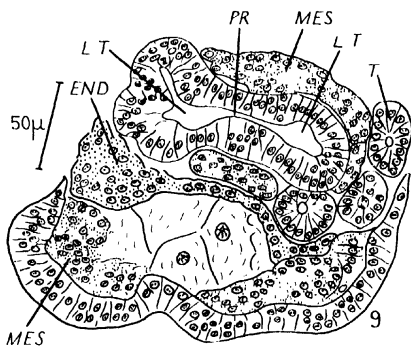
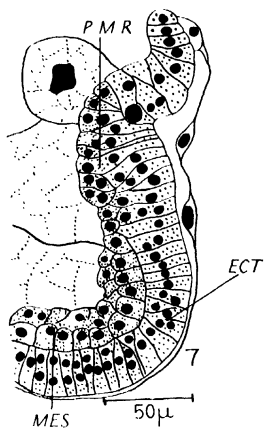
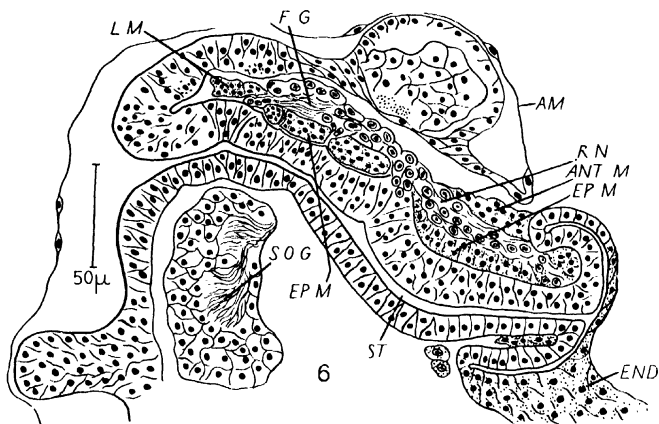
Fig. 7.—Longitudinal sagittal section through the posterior end of a 30-hour embryo. The mesendoderm rudiment is shown immediately above the site of appearance of the proctodaeum.

Fig. 8.—Same in a 48-hour embryo. The proctodaeum has now passed inwards and endoderm and mesoderm are completely separated.

Fig. 9.—An obliquely transverse section across the inner end of the same stage as fig. 8. On the left of the figure the origin of the Malpighian tubules from a lateral lobe is shown. On the right the three tubules are seen passing backwards. The endoderm is also seen to be in the form of a pair of lateral masses connected by a thin median plate or strand.

Fig. 10.—A coronal longitudinal section of the proctodaeum in a 30-hour embryo.





On the skeleton of the hyoid arch in Rays and Skates.

By

G. R. de Beer

With Plates 19-21 and 1 Text-figure.

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INTRODUCTION.

IN 1926 I investigated the development of the skull in *Torpedo*, and, on paying attention to the skeleton of the hyoid arch, I came to the conclusion that the interpretation placed upon it by Gegenbaur (1872), and commonly held, is incorrect. Briefly, this view is that the cartilaginous arch bearing hyal rays, which in the Batoidei is situated behind the hyomandibula, represents the hyoideum or ceratohyal which has become disconnected from the hyomandibula and has extended upwards (dorsally) behind it. To this I preferred Krivetski's (1917) conclusion to the effect that the cartilaginous arch in question represents the fused bases of the hyal rays, and that it should therefore be termed a pseudohyoid, to distinguish it from the ceratohyal of other forms with which it is not homologous. This conclusion is based on the fact that the afferent pseudo-branchial artery, on running forwards from the efferent hyoidean artery, is median to the pseudohyoid, whereas it is lateral to the ceratohyal of the non-Rajiform Selachians. It may be noted that in the latter forms¹ it is common for a number of

¹ There appears to be no convenient collective term for the non-Rajiform

hyal rays to be fused together at their base, forming little bars, and the afferent pseudobranchial artery is median to them. The morphological relations between the cartilages briefly referred to above are plainly evident in Dohrn's (1886) figures, in Krivetski's (1917), and in my own (1926).

In a recent paper, however, Edgeworth (1931) has denied the truth of these statements, referring to *Torpedo ocellata* with the words, 'there is no vessel passing internal to the lower part and external to the upper part of the hyoid bar', which he repeats in respect of *Raja clavata*. But the figures which Edgeworth gives to illustrate his paper flatly contradict his text, and the artery in question (afferent pseudobranchial), called by him afferent mandibular artery, is shown by him in *Torpedo ocellata* morphologically internal or median to the lower part of the bar in his figs. 2, 3, 4, 5, and 11, and morphologically external or lateral to the upper part of the bar (or hyomandibula) in figs. 9 and 10. On the other hand, in *Scyllium canicula*, Edgeworth shows that the afferent pseudobranchial artery passes lateral or external to the whole of the hyoid bar, i.e. to both hyomandibula and ceratohyal, in his figs. 17, 18, 19, 20, 22, 23, and 24.

As they stand, Edgeworth's figures are sufficient to confute his opinion and to substantiate the contrary one to the effect that while the upper part of the hyoid bar or hyomandibula is the same in Sharks and Rays, the lower part is not the same in its morphological relations in the two groups. He says further that, 'in an embryo of 25 mm. the hyoid bar has separated into the hyomandibula and the hyoideum'. I have not been able to reconcile this statement with my preparations, and, in view of the confused state of the problem and the conflict of evidence in regard to it, I have thought it advisable to reinvestigate the matter, and to present a few microphotographs. It may be said at once that the result of this work has been to confirm Krivetski's and my view completely.

In the preparation of the microphotographs I enjoyed the help
Selachians other than the comparatively little used *Pleurotremata*: the term *Squaliformes* is used for a particular suborder of sharks. In the present paper they will be referred to simply as 'Sharks', in contradistinction to 'Rays'.

of Messrs. P. A. Trotman and W. Chesterman. I wish to record my appreciation of their services, and particularly to acknowledge my gratitude to Professor E. S. Goodrich, F.R.S., for the excellent facilities which I have enjoyed in his Department of Zoology and Comparative Anatomy of the University Museum, in which the work recorded in this paper was done.

The material consisted of series of sections of *Torpedo ocellata* and *marmorata* of sizes varying from 12 mm. to 30 mm., of sections of *Raja blanda* (young), and of specimens of young and adult *Rhynchobatus* and of young *Pristis* which were studied by dissection. For access to the latter I am indebted to the kindness of my friend Mr. J. R. Norman, of the British Museum (Natural History).

OBSERVATIONS.

The morphological point at issue concerns the relations of certain cartilages to an artery, the afferent pseudobranchial artery, and as a preliminary it is necessary to consider the nature of the latter.

The visceral arches of the Selachians each contain typically two efferent arteries which are interconnected about half-way up the arch by a short commissural artery which is external or lateral to the cartilaginous skeleton of the arch, and internal or median to the afferent artery and to the main trunk of the dorsal nerve corresponding to the arch. These relations hold good for all the branchial arches, as well in the Rays as in the Sharks. The first branchial arch of *Scyllium canicula* is shown in fig. 12, Pl. 20, and that of *Torpedo ocellata* in fig. 9, Pl. 20, as seen in transverse section; the first branchial arch of *Torpedo marmorata* is shown in fig. 13, Pl. 21, and that of *Raja blanda* in fig. 14, Pl. 21, as seen in horizontal section, from which the morphological relations described above can be clearly made out.

The hyoid arch differs from the more posterior visceral arches in that it contains only one (posterior) efferent artery. But at the same level as the commissural arteries of the branchial arches, the efferent hyoid artery gives off the afferent pseudo-branchial artery. That the latter is serially homologous with

that while they are formed as parts of the hyoid bar which is internal to the afferent pseudobranchial artery, they are quite distinct from the pseudohyoid, which is external to that artery. The pseudohyoid is now chondrified to a certain extent, and its extremities are attached by mesenchyme, the upper to the hindmost part of the hyomandibula, and the lower to the hyoid bar in the region of the ceratohyal. Some of the hyal rays are also cartilaginous, and they appear to be continuous with the pseudohyoid, even when studied with the help of a critical cartilage-stain such as thionin.

The ceratohyal is, therefore, not entirely absent in the Rays, but it is very much reduced. It may be noted that it bears the correct relations to the afferent hyoid artery, being immediately dorsal to it. It is connected with the hyomandibula only by a string of mesenchyme, and lies at a level 0.3 mm. behind the hyomandibula. To the strand of mesenchyme between the hyomandibula and the ceratohyal there is attached a ligament which passes backwards, inwards, and downwards, dorsally to the ceratohyal, the hypohyal, and the afferent hyoid artery, to join a large paired muscle which extends backwards dorsally to the coracomandibular muscles and ventrally to the ventral aorta and to the coracobranchial muscles. This ligament is seen in figs. 3, 5, 6, Pl. 19, and fig. 7, Pl. 20, and the muscle to which it is attached in figs. 8 and 9, Pl. 20. The muscle appears to be that which Marion (1905) described in *Raja* as the coracohyomandibular. It is not an ordinary coracohyoid muscle, for it is dorsal to the afferent hyoid artery and afferent first branchial artery, instead of ventral to them as the coracohyoid muscle is in Sharks. Luther (1909) has, however, described some dorsal fibres of what he calls the coracohyoid in *Stegostoma*.

In addition to the coracohyomandibular ligament and muscle, the hyoid bar comes into relations with a part of the second superficial constrictor muscle (C_2 hv according to Ruge's (1897) terminology) which in *Raja* forms the depressor hyomandibularis muscle. This is seen in figs. 1, 3, 5, Pl. 19, and fig. 7, Pl. 20. This muscle is stated by Ruge to be wanting in *Torpedo*, whereas Tiesing (1896) speaks of it as present. According to Fürbringer (1897) the coracohyoid muscle of *Raja* is

separated from the depressor hyomandibularis by the first afferent branchial artery. Since the coracohyoid muscle must be ventral (superficial) to the artery, it follows that the depressor hyomandibularis must be dorsal to (deeper than) the artery, and so it would seem that the muscle which Fürbringer calls the depressor hyomandibularis must occupy the same position as that muscle in Raja which Marion (1905) has called the coracohyomandibular. Altogether, the question of the muscles of the hyoid arch in the Rays appears to be distressingly confused, as Allis (1917) has already pointed out.

Later stages of development show little novelty, but chondrification of hyomandibula, ceratohyal, hypohyal, pseudohyoid, and hyal rays soon becomes complete (figs. 5 and 6, Pl. 19, and figs. 7 and 8, Pl. 20). It is worth noting, as well seen in fig. 8, that the ceratohyal is at a deeper level than the pseudohyoid. With regard to the hyal rays it may be mentioned that Edgeworth (1931) maintains that they are originally independent of the bar (pseudohyoid) to which they are fused. All that I can say is that I have not observed the separate nature of the rays situated at about the middle of the arch, and a study of sections and of whole preparations stained by the van Wihje technique leads me to believe that these rays at any rate are continuous with the pseudohyoid. I have, however, seen that the more dorsal and ventral of the hyal rays do seem to possess separate centres of chondrification, and this leads to a consideration of the nature of the pseudohyoid itself.

Krivetski (1917) regarded the pseudohyoid as formed by the fusion of the proximal ends of the hyal rays, and it is therefore necessary to turn now to the Sharks in order to see whether their hyal rays show any trace of such a fusion. Fürbringer (1903), who devoted a study to the visceral skeleton of the Sharks, showed that fusion of the hyal rays occurred to a greater or lesser extent in a number of forms, such as *Chlamydoscelachus*, *Odontaspis*, *Heterodontus*, *Spinax*, *Echinorhinus*, *Laemargus*, and *Scymnus*. Luther (1909) demonstrated a very extensive fusion in *Stegostoma*, and I have observed it in *Carcharodon* and various species of *Scyllium*. Fig. 10, Pl. 20, is of a transverse section through

the posterior part of the hyoid arch of an embryo of *Scyllium canicula*. The section is taken behind the level of the hyomandibula and ceratohyal, but a number of hyal rays are cut, and it may be observed that five of the most ventral of the dorsal set of hyal rays are fused together forming a bar, and three of the most dorsal of the ventral set of rays are likewise fused. But most important of all it must be noticed that the bars formed by this fusion are lateral or external to the afferent pseudobranchial artery. In fact, these bars occupy precisely the same morphological position as the pseudohyoid of the Rays, and it would be very extravagant in hypotheses to suppose that they were not homologous. In the Sharks these bars, which may be termed the pseudohyoid bars, remain separate: a dorsal one and a ventral one. In the early stages of *Torpedo*, whole preparations stained with Victoria blue show that they have separate centres of chondrification: a little later, however (fig. 8, Pl. 20), they are joined into a single rod. Whether they remain so joined in the adult condition in *Torpedo* I do not know, but in adult *Raja*, *Rhynchobatus*, and *Pristis* they are separated into dorsal and ventral portions with a joint between.

A question now arises as to the manner in which the fusion of the hyal rays has taken place. Fürbringer, Luther, and Krivetski speak of a fusion or conerescence of the proximal ends of the hyal rays. In this way it can be imagined that the pseudohyoid bar might be formed, like the cross-piece which bears the pegs of a rake. But there is another way in which it may be imagined that the process has taken place. It will be noticed that the rays which are so fused in the Sharks are the central ones, the most dorsal and most ventral rays being free. I may also repeat that it is the central rays which I have found to be continuous in their chondrification with the pseudohyoid, while the more dorsal and more ventral rays seem to possess separate centres of chondrification. This looks as if in the formation of the pseudohyoid bars the first step was the elongation and enlargement of the most ventral ray of the dorsal series and the most dorsal ray of the ventral series. On to these elongated rays the bases of the adjacent rays would be fused

in succession, in a dorsal direction for the dorsal set of rays and in a ventral direction for the ventral set. While the rays nearest the middle of the hyoid arch (the central rays) may be supposed to chondrify with the pseudohyoid bar—indeed, on this view each pseudohyoid bar itself would be an elongated ray—the more dorsal and more ventral rays may retain a certain amount of independence in chondrification.

The study of developmental stages of *Torpedo* has shown, therefore, that the ceratohyal is not absent but simply very much reduced; that the pseudohyoid is an element quite separate from the ceratohyal; that the pseudohyoid is formed by the fusion of hyal rays, probably on to enlarged and elongated rays; and comparison with Sharks has shown that such a fusion in them is the rule rather than the exception.

It may now be asked whether the ceratohyal is present in other Rays besides *Torpedo*, and the answer is that it is. A study of sections of *Raja blanda* has revealed the existence of a small elongated splint of cartilage at each end of the hyoid copula, and median to the ventral end of the pseudohyoid of its side. A similar cartilage has been found in preparations of *Pristis*, but the most interesting condition of all is that shown by *Rhynchobatus*. This form is regarded as one of the most primitive of the Rays, and it might be expected therefore that the ceratohyal in it was not so much reduced as it is in *Torpedo*, *Raja*, or *Pristis*. Fig. 15, Pl. 21, is a view of the hyoid copula and pseudohyoid of *Rhynchobatus*, and in between these cartilages the ceratohyal can be plainly seen, and it presents evidence of its more primitive condition in that it still bears four hyal rays: all the other rays are borne by the pseudohyoid. It may also be noticed that the ceratohyal is composed of denser cartilage than the pseudohyoid, and, indeed, the latter shows a number of circular zones of incomplete chondrification, indicative of the newness of its formation.

DISCUSSION.

By the recognition of the pseudohyoid of the Rays as a structure altogether distinct from the ceratohyal or hyoideum and formed by the fusion of the hyal rays, all the morphological

relations of these structures can be recognized as having been respected during the phylogenetic divergence between the Rays and the Sharks. Further, the presence of a ceratohyal in the Rays, separate from the pseudohyoid, and the presence of pseudohyoid bars in the Sharks, separate from the ceratohyal, renders it quite impossible to regard these structures as the same. In addition, this view solves the problem of why the pseudohyoid extends dorsally behind the hyomandibula and reaches a point more dorsal than that which the ceratohyal normally attains.

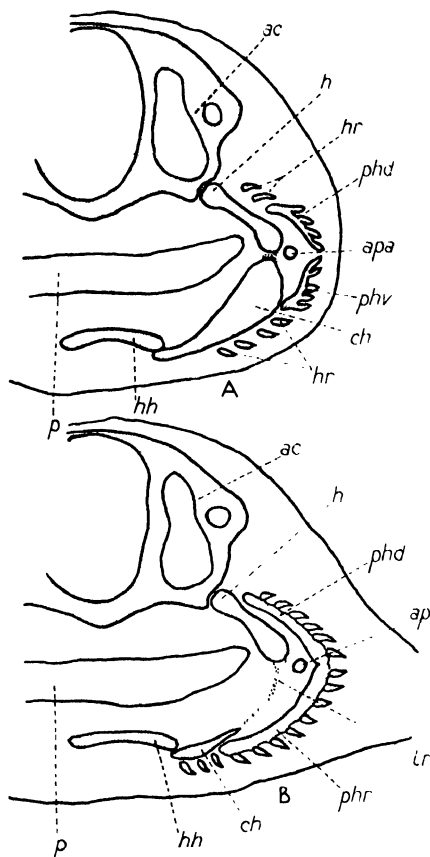
It is not without interest to note that the formation in the Rays of a pseudohyoid bar by fusion of hyal rays is a phenomenon which seems to have a parallel in the Dipnoi. Fürbringer (1904) has drawn attention to the conditions in *Protopterus*, where there is a cartilaginous rod between the hyoid and first branchial arch. He supposes that it has been formed by the fusion of the bases of rays, and he also provides a reason for this modification.

The anterior wall of the first gill-slit (second visceral cleft, referred to by Edgeworth as '2nd gill-cleft') is supported by the hyal rays, and provided that the distance between this slit and the angle of the mouth be not too great, the hyal rays can themselves be supported on the hyomandibula and ceratohyal; if not, the rays must have some other support. This argument is all the more cogent in the Rays, where, in the first place, the hyomandibula is tied to the angle of the mouth in consequence of its hyostylic function, and where the gill-slits have become separated by a considerable distance from the mouth and spiracle: the spiracle being on the dorsal side of the body while the gill-slits are on the ventral side. The normal skeleton of the hyoid arch is therefore incapable of supporting the hyal rays under such conditions, and the last vestige of this function on the part of the ceratohyal is seen in *Rhynchobatus*.

In the light of the present work, the relations of the skeleton of the hyoid arch in Sharks and Rays is shown diagrammatically in Text-fig. 1.

In addition to the particular conclusions to which this work has led, there may be derived from it some considerations of

more general value and application. Whether the work had been begun by a study of the anatomy of the skeleton of



TEXT-FIG. 1.

Diagrammatic representation of the relations of the skeletal structures of the hyoid arch in *A*, the Sharks, *B*, the Rays.

Rhynchobatus with its little ceratohyal still bearing rays, or of the embryology of *Torpedo* with its distinct hyoid and pseudohyoid bars, or of the morphology of the hyoid arch skeleton in the Selachians with the discrepancy between the relations of the afferent pseudobranchial artery to the ceratohyal

and pseudohyoid, its results would have pointed in the same direction, each line of work confirming the others. Indeed, it was because of evidence from morphology of the hyoid arch skeleton in Selachians and embryology of *Torpedo* that *Rhynchobatus* was suspected of having a ceratohyal which still retains its function of bearing hyal rays. This amounts to saying that morphological conclusions, if correct, have more than a merely descriptive value, for they enable predictions to be made, and these may be susceptible of positive verification if suitable material be available in which to test them. Investigation of such material then becomes an 'experiment', of no less philosophical value than in the so-called experimental branches of science: a fact which seems to be in danger of being lost sight of. The only handicap from which morphology suffers is the fact that, owing to the widespread extinction which has accompanied evolution, suitable material may not be available, and there may consequently be many hypotheses incapable of proof.

SUMMARY.

1. The relations to the afferent pseudobranchial artery of the lower ray-bearing part of the hyoid arch skeleton in Rays show that the latter is not the ceratohyal, but a pseudohyoid.

2. Investigation of the development of *Torpedo* shows that the pseudohyoid arises distinct from the hyoid bar, and that the ceratohyal is much reduced.

3. The ceratohyal in *Rhynchobatus* is not so much reduced as in *Torpedo*, *Pristis*, or *Raja*, and it still bears a small number of hyal rays.

4. The pseudohyoid is formed as the result of fusion of hyal rays, and pseudohyoid bars are present more or less well developed in a large number of Sharks.

5. The enlargement of the pseudohyoid and reduction of the ceratohyal in Rays is associated with the increased distance between the mouth and the first gill-slit, and the necessity for providing a support for the hyal rays and the anterior wall of the first gill-slit. Similar factors have been operative in the Dipnoi and have led to analogous results.

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EXPLANATION OF PLATES 19, 20, AND 21.

PLATE 19.

Fig. 1.—Transverse section through an embryo of *Torpedo ocellata* 21 mm. long (section 21-4-3-6), showing the relations of the hyoid bar to the afferent pseudobranchial artery.

Fig. 2.—Ditto, 0.2 mm. posterior to fig. 1 (section 21-4-4-3), showing the relations of the pseudohyoid bar to the afferent pseudobranchial artery.

Fig. 3.—Transverse section through an embryo of *Torpedo ocellata* 22 mm. long (section 22-4-2-5).

Fig. 4.—Ditto, 0.3 mm. posterior to fig. 3 (section 22-4-3-8).

Fig. 5.—Transverse section through an embryo of *Torpedo ocellata* 27 mm. long (section 27-5-3-10).

Fig. 6.—Ditto, 0.3 mm. posterior to fig. 5 (section 27-6-1-5).

PLATE 20.

Fig. 7.—Transverse section through an embryo of *Torpedo ocellata* 30 mm. long (section 30-6-1-8).

Fig. 8.—Ditto, 0.32 mm. posterior to fig. 7 (section 30-6-3-5).

Fig. 9.—Transverse section through an embryo of *Torpedo ocellata* 22 mm. long (section 22-5-2-6), showing the relations of the skeleton of the first branchial arch to the cross-commissural artery.

Fig. 10.—Transverse section through an embryo of *Scyllium canicula* 35 mm. long (section 35-7-2-5), showing the fusion of hyal rays to form dorsal and ventral pseudohyoid bars.

Fig. 11.—Ditto (section 35-6-3-6), showing the relations of the hyoid arch skeleton to the afferent pseudobranchial artery.

Fig. 12.—Ditto (section 35-7-4-3), showing the relations of the skeleton of the first branchial arch to the cross-commissural artery.

PLATE 21.

Fig. 13. Horizontal section through an embryo of *Torpedo marmorata* 24 mm. long (section 24-10-2-8), showing the various relations of the cartilages to the arteries and nerves.

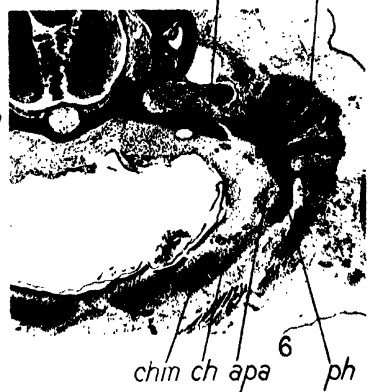
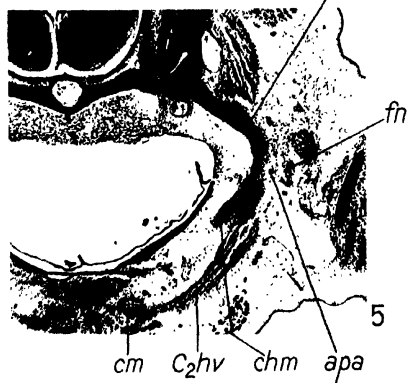
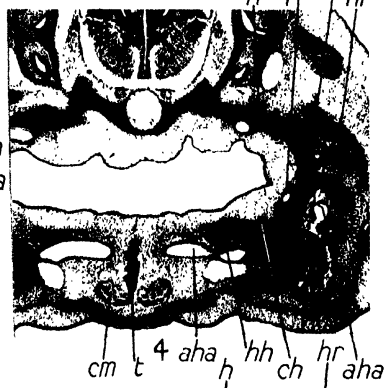
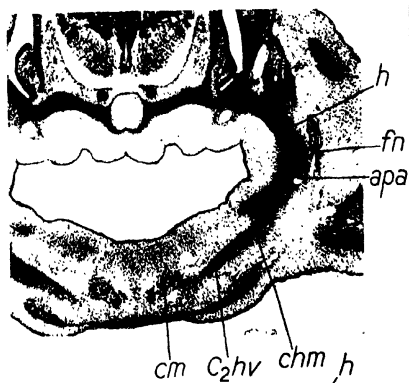
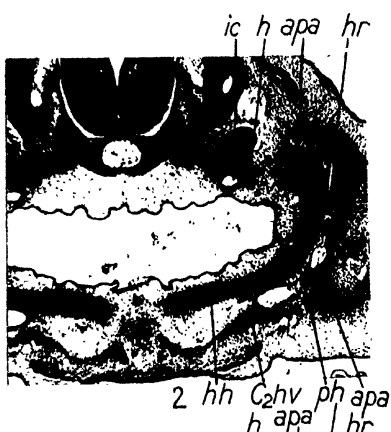
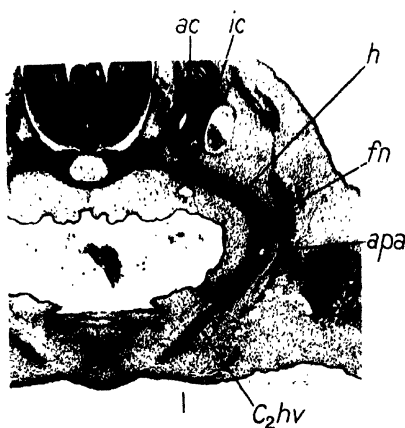
Fig. 14.—Horizontal section through a young specimen of *Raja blanda* (section 113), showing the various relations of the cartilages to the arteries.

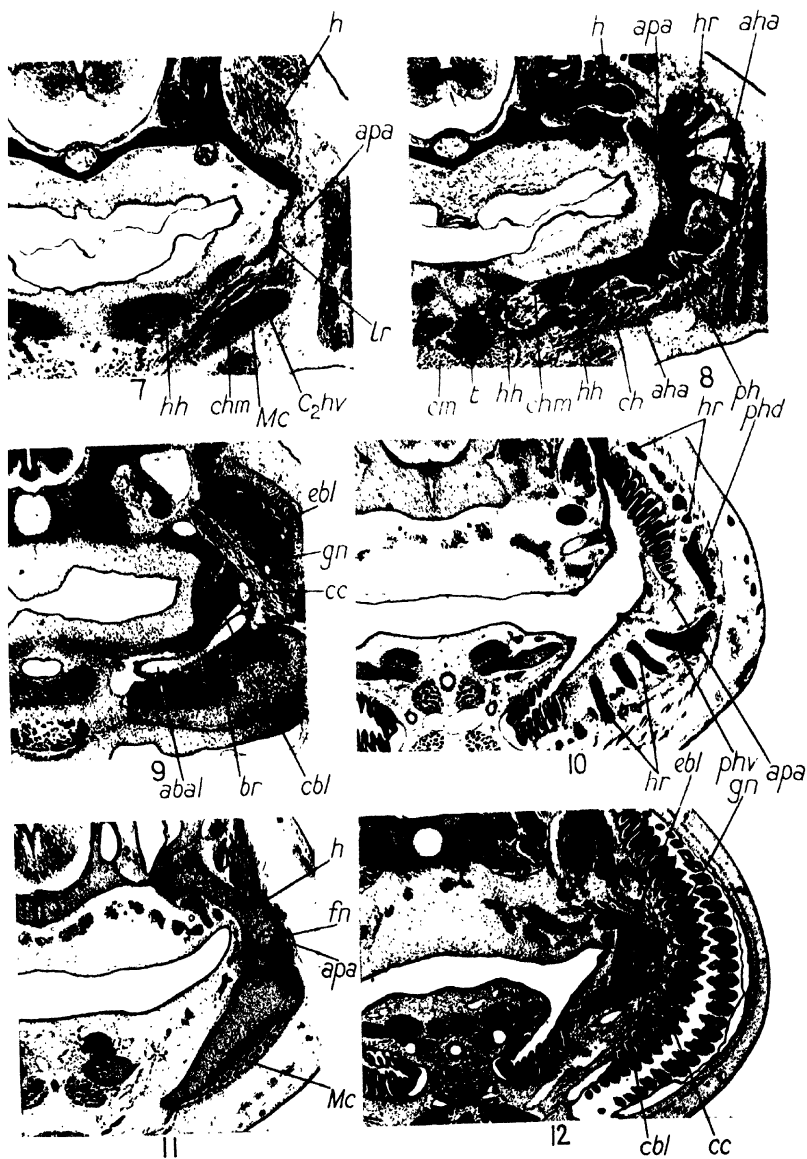
Fig. 15.—Photograph of a preparation stained with Victoria blue of the skeleton of the left hyoid arch of *Rhynchobatus*, showing the hyoid copula, the ceratohyal bearing hyal rays, the pseudohyoid divided into dorsal and ventral portions, and a small portion of the dorsal extremity of the hyomandibula.

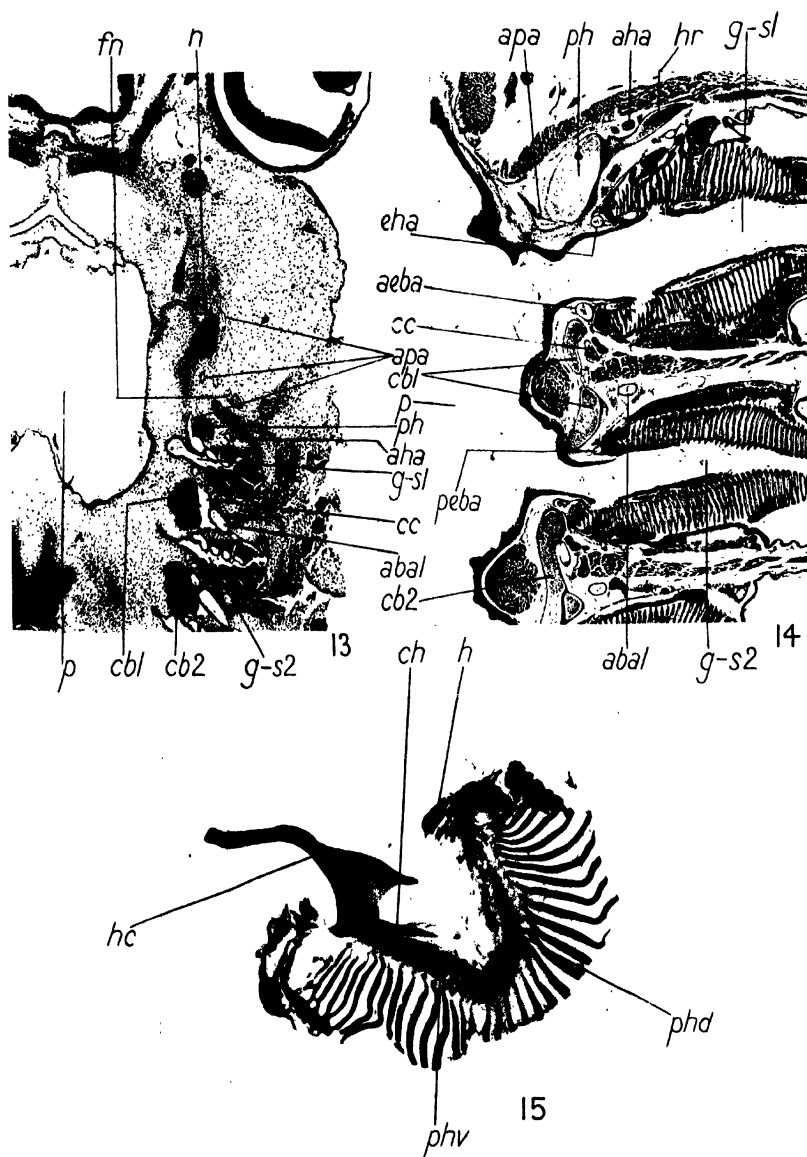
EXPLANATION OF LETTERING.

aba, 1 and 2, first and second afferent branchial artery; *ac*, auditory capsule; *aeba*, anterior efferent branchial artery of first branchial arch; *aha*, afferent hyoid artery; *apa*, afferent pseudobranchial artery; *br*, branchial ray; *cb*, 1 and 2, first and second ceratobranchial; *cc*, cross-commissural artery between anterior and posterior efferent branchial arteries; *ch*, ceratohyal; *chm*, coracohyomandibular muscle or ligament; *C₂hn*, posteroventral portion of second constrictor muscle; *cm*, coracomandibular muscle; *eb*, 1, first epibranchial; *cha*, efferent hyoid artery; *fn*, facial nerve; *gn*, glossopharyngeal nerve; *g-s*, 1 and 2, first and second gill-slit; *h*, hyomandibula; *hc*, hyoid copula; *hh*, hypohyal; *hr*, hyal ray; *ic*, internal carotid artery; *lr*, ligamentous vestige of hyoid bar between hyomandibula and ceratohyal; *Mc*, Meckel's cartilage; *p*, cavity of pharynx; *peba*, posterior efferent branchial artery of first branchial arch; *ph*, pseudohyoid; *phd*, dorsal pseudohyoid bar; *phv*, ventral pseudohyoid bar; *t*, thyroid gland.

Erratum: in fig. 2, Pl. 19, bottom right-hand corner, for *apa* read *aha*.







The Loxosomatidae of the Plymouth Area, including *L. obesum* sp. nov.

By

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With 24 Text-figures.

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INTRODUCTION.

Four known species of *Loxosoma*, namely, *L. phascolosomatum* Vogt, *L. crassicauda* Salensky, *L. singulare* Keferstein, and *L. claviforme* Hincks, have been identified as occurring in the Plymouth area, and a new species *L. obesum* is described.

Observations were made so far as possible on living material. Text-figures (except Text-figs. 6, B, and 8, A) of living *Loxosomas* are of individuals narcotized with stovain, and in such individuals the tentacular crown is generally more widely open than is normal. *L. crassicauda* was the easiest successfully to narcotize, and *L. obesum* the most difficult. Measurements, unless otherwise stated, are of living narcotized specimens with the tentacles expanded. Total length is measured from the disc of attachment, or the end of the stalk, to the edge of the lophophore, between the bases of the two most dorsal tentacles.¹ The tentacles were not included, so that the measurement should be roughly comparable with that of specimens with closed lophophore.

The only commensal of *Loxosoma* seen was a species of *Lienophora* on two individuals of *L. crassicauda*. About ten specimens were present on each, most of them being on the dorsal surface of the calyx. This Infusorian occurs in numbers on *Diplosoma* living in the same tanks in the Laboratory as *L. crassicauda*, and its presence on the Polyzoan was probably accidental.

LOXOSOMA PHASCOLOSOMATUM Vogt.

This well-known species is found in the Salcombe Estuary growing on the caudal extremity of *Phascolosoma vulgare* (see also 'Journ. Mar. Biol. Assoc.', vol. vi, N.S., p. 164, 1900), and in addition on *Lepton clarkiae* and *Montacuta bidentata*,² two tiny Lamellibranchs occurring in

¹ The terms 'dorsal' and 'ventral' are used in accordance with the interpretation of the relations of the body given by Harmer for *Pedicellina* on p. 261, 'Quart. Journ. Micr. Sci.', vol. xxvii, 1886 (and not for *Loxosoma*, on p. 264, 'Quart. Journ. Micr. Sci.', vol. xxv, 1885). The mouth is nearer the 'ventral' side, and the anus nearer the 'dorsal' side.

² *Mysella bidentata* in the Plymouth Marine Fauna, 1931.

the burrows of *Phascolosoma* (*pellucidum*) *elongatum*. A short account of this species has already been given by me (2), with figures mostly of living specimens.

LOXOSOMA CRASSICAUDA Salensky (Text-figs. 1-3).

Habitat.

In February 1929 specimens of *Loxosoma crassicauda* Salensky¹ were found growing on the wall of a shallow wooden table-tank, placed under windows facing south in the Plymouth Laboratory. In August of the same year this species was discovered attached to several different kinds of worm-tubes, including *Sabella*, *Branchiomma*, and *Bispira*, and to clean pebbles in a small tank in the Aquarium. It has since been found on the walls of two of the large tanks in the Laboratory. Batches of *Loxosomas* from the shallow tank were examined about once a month up to February 1930. On September 18, 1929, only four medium-sized individuals (0.75-1.0 mm. long) could be found, though thirty-three had been easily obtained on August 23; by the end of October, however, specimens were again obtainable. Possibly many *Loxosomas* may have died in the intervening time owing to the high temperature reached by the water in the shallow tank (e.g. on September 11 a temperature of 20-1° C. was recorded).

Although the tubes of *Phyllochaetopterus socialis* Clap.² (probably the original host of *L. crassicauda*, see 32) have been examined, so far they have not yielded *Loxosomas*.

L. crassicauda was first discovered by Salensky (29, p. 2) in the spring of 1874 at Naples inhabiting the 'coquilles tuberculeuses' of an annelid which he was unable to identify. Schmidt (32, p. 71) in the spring of 1877 found a species on the tubes of *Phyllochaetopterus socialis* Claparède, which he says completely agreed with Salensky's description and

¹ It has been considered advisable to retain the generic name of *Loxosoma* for this species, and not to place it in the genus *Loxosomella* Mort., of which Mortensen (19, p. 405) makes it the genotype. In this connexion see Harmer, 12, p. 6.

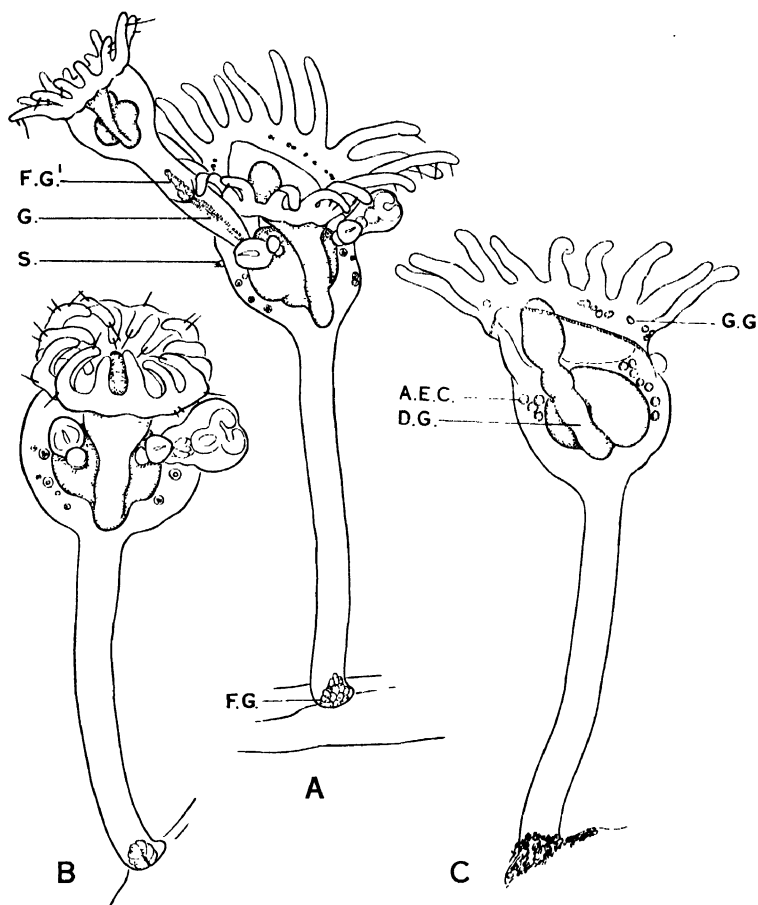
² In Plymouth Marine Fauna, 1931, as *P. anglica* Potts.

figures, except that a foot-gland was present in the adult. This species was later found by Harmer in 1885 (9, p. 263) in large numbers attached to the floor of a tank in the Zoological Station at Naples, and since has been doubtfully identified by Kirkpatrick as growing on algae from the Tizard Reef in the China Sea (17, p. 23). Sir S. F. Harmer informs me by letter that in 1903 he found very numerous specimens of *L. crassicauda* on the test of *Ciona intestinalis* and on the Polyzoan, *Zoobotryon pellucidum*, growing on the *Ciona*, in a store-bottle (from Naples) in the Zoological Laboratory at Cambridge.

The record of *L. crassicauda* at Plymouth would appear to be the first for the British Isles.

Notes on the Morphology.

L. crassicauda at Plymouth may reach a length of 1.87 mm. Measurements of sixty specimens gave an average total length of 1.4 mm., with length of calyx 0.5 mm., and of stalk 0.9 mm.; the average width of the calyx was 0.38 mm. The stalk of adult individuals is between 0.06 to 0.1 mm. wide. *L. crassicauda* (Text-fig. 1) is a large, transparent species; the calyx is rather broad, the stalk long and slender, the one passing somewhat abruptly into the other. The termination of the stalk is more or less cylindrical; a reduced foot-gland is usually present in the adult. The lophophore is large, the number of tentacles in the adult of the Plymouth form being 15, 16, or 17; usually 16. In the number of tentacles, and the retention of a reduced foot-gland in the majority of adults examined, it differs from those described by Salensky (29) and Harmer (9, p. 263) of which the number of tentacles is said to be typically 18, though by no means constant, and a foot-gland is said to be absent in the adult. Salensky mentions that of the 18 tentacles 2 are rudimentary (29, p. 3), and in his fig. 1, Pl. 12, only 17 are shown. The smaller number of tentacles in the Plymouth specimens would not appear to be an important difference in a species where the number undergoes a progressive increase from that present in the bud on liberation; and, as Schmidt (32, p. 72) has recorded, the foot-gland in the



TEXT-FIG. 1.

L. crassicauda. Living individuals. $\times 57.25$. A. Ventral view with lophophore fully expanded. B. Ventral view with lophophore partly closed. C. Sketch showing dorsal surface of animal. Large cells (A.E.C.), possibly excretory or accretory in function, are present on either side of the alimentary canal; in this individual they were clear and not granular in appearance. D.G., dorsal groove connecting the apical region of the stomach with the intestine; F.G., foot-gland of adult; F.G.¹, foot-gland of bud; G., groove of foot-gland; G.G., large granular gland-cells; S., sense-organ. The large transparent vacuolated glands are not visible at this magnification, unless stained intra-vitam with neutral red, and so are not shown in the figure.

adult is indistinct and is often obscured by dirt particles collected round the point of attachment; it generally shows clearly only in stained and mounted specimens. Schmidt says that the gland is of the same nature and size as in the buds, but in the individuals examined it was found to vary in size; in most it was smaller than in the bud, in others it appeared to be breaking down, while in a few it was absent. Actual figures are: out of 81 stained and mounted specimens in which the end of the stalk could be clearly seen, 63 had a distinct gland, in 7 it was tiny, in 5 it was breaking down, and in 6 it was absent. The specimens varied in size from young attached forms 0.8 mm. in length to adults up to 1.87 mm. It is probable that after the bud becomes attached the foot-gland loses its power to secrete and slowly atrophies.

The Plymouth form has the characteristic paired sense-organs of *L. crassicauda*; their tuft of stiff hairs is about 45μ long. The sense-organs are of a good size in buds only 0.3 mm. in length attached to the parent: they do not appear to be retractile as are those of *L. phascolosomatum* (36, p. 313). Duplication of the sense-organ of one side occurred in an individual, the nerve from the ganglion dividing on approaching the edge of the calyx.

The tactile hairs on the outer, non-ciliated part of the tentacles are particularly long and conspicuous in this species, both in the adult and the bud. In well-developed buds there is usually a stiff sense-hair on either side of the aperture of the foot-gland at the 'heel' of the foot (see Text-fig. 2, B). The pore of the gland opens into the extreme distal end of the groove which traverses the 'sole' of the foot.

Numerous large gland-cells of the two types described by Harmer (9, p. 266) occur in two rows parallel to the edge of the vestibule and of the calyx. The opaque granular-looking glands are somewhat pear-shaped, about 15μ to 34μ long, and occur at irregular intervals; the transparent glands, filled with large vacuoles, are considerably larger, and may be $45\mu \times 27\mu$, with vacuoles up to 13μ in diameter; these occur in a rather regular row slightly dorsal to the former gland-cells. When a slight trace of neutral red is added to the sea-water containing the

Loxosomas, the transparent vacuolated glands, by reason of their end vacuoles taking the stain, show up in a conspicuous row, especially regular in well-developed buds. The small vacuoles near the external aperture of each gland (Harmer, 9, p. 266, found the glands to open externally) alone take the neutral red, becoming bright, slightly orange red, while those filling the major half of the gland are either tinted extremely faint pink or are colourless. These glands take up the colour rapidly, the granules round the nuclei of the ectoderm cells alone being coloured before them: the colour remains some days after the animals are returned to clear sea-water. The granular gland-cells, on the other hand, only show one or two granules in the centre slightly coloured after twenty-four hours or more. Both types of gland-cell are evidently not mucus glands, as they are unstained by Mayer's mucuacematein.

A group of large, rather deep-seated cells about 22μ – 27μ in diameter occur in the calyx on either side of the oesophagus (see Text-fig. 1, c). They are generally, though not always, granular in appearance, and in most instances have a definite large granular central mass, or two or three central granules, yellow in colour. The number of these cells varies, but is most commonly four or five on each side, sometimes arranged more or less in linear series, sometimes in a loose cluster; under a high magnification they are seen to be separate from one another in the living animal. Occasionally one or more cells similar in character occur more proximally close to the side-walls of the stomach, but may be separated from the distal group by a considerable space. They appear to increase in number with age; they perhaps have an excretory function, or rather waste products are stored in them. These cells are in the position of Salensky's 'glandules multicellulaires ayant la forme de deux grappes', but ducts have not been distinguished opening to the edge of the calyx as he describes and figures (29, pp. 10 and 11, and Pl. 13, fig. 14); it is perhaps possible that he mistook some of the numerous nerves for ducts. He describes them as composed of transparent protoplasm—this may be a phase in their activity—and suggests that they may be 'glandes rénales' (29, p. 11). These paired clusters of cells are roughly in the

position of, and somewhat similar in character to, those described by various authors in other species as excretory in character (lophophore and body kidneys of *L. saltans* (1, p. 132), and both groups of excretory organs of *L. davenporti* (23, p. 372) and *L. annelidicola* (27, p. 100)), but they are distinct from the true nephridia studied in *L. crassicauda* by Harmer (9, p. 277), and Stiasny (34, p. 192), and of which the wave-like motion of the cilia lining the duct, or perhaps of the flagellum of the flame-cell itself, can be seen. When a trace of neutral red is added to the sea-water, these cells show colour fairly soon; at first it is as a diffuse pink surrounding the yellow granular central mass; later the granular centre takes the colour darkly, becoming dirty, deep, orange red, the original yellow colour no doubt affecting the tone of the red. The colour remains some days after the animals are returned to clean sea-water. When the sea-water is tinted with methylene blue these cells become pale blue, with dark-blue centres, and retain their colour for twelve hours or more. In sections stained with iron haematoxylin and acid fuchsin, after fixation in strong Flemming's fluid without acetic acid, these cells are conspicuous; they show a darkly staining cytoplasmic border, in which is the nucleus, and a granular centre, generally separated by a ring which does not take the stain.

The ectoderm cells of the stalk tend to be arranged in longitudinal rows, especially in young individuals. This is possibly a variable character, for while Salensky (29, p. 8) speaks of a longitudinal row of cells, which he took to be glandular, down the dorsal side of the stalk, Harmer (9, p. 263) states that there was no regular arrangement of the ectoderm cells in his specimens. Longitudinal muscles only occur in the stalk, but they are sufficiently well developed to allow the animal to throw itself about with irritable violence if touched, the lophophore bending to the base of the stalk.

The alimentary canal is of the normal type. Just proximal to the so-called liver-lobes there is a slight development of the lobes, which Assheton (1, p. 129) suggested for *L. saltans* had a secretory function, in distinction to the 'liver'-lobes, the characters of which he considered indicated active constructive

metabolism. The cells of the 'liver'-lobes in adult *L. crassicauda* are about 0.025–0.05 mm. deep. The rectum when packed with waste matter is particularly large; after the expulsion of faeces, however, it collapses. Its walls are frequently crowded with large, shining, yellowish spherules and small granules. Assheton considered in the case of *L. saltans* (1, p. 133) that the rectum was in all probability an important part of the excretory system. Sections show some specimens with deep rectal cells, others with shallow cells.

The whole of the alimentary canal is ciliated, as stated by Harmer (9, p. 276), but the cells of the lateral diverticula of the stomach may lose their cilia when actively secreting or excreting. Waste matter collected in the dorsal groove of the stomach and in the intestine may be seen revolving, but if the watch-glass containing the animal is jerked, the motion may cease in the intestine for five minutes or more, and then begin again slowly, gradually gathering speed. The stoppage of movement is probably due to muscular contraction causing reduction of the lumen, and so preventing the cilia working efficiently, and not to cessation of beat of the cilia clothing the walls of the intestine. Reversal of the rotation of the mass of food-particles in the intestine and dorsal groove occurs, as described by Cori (6, p. 37) in the stomach of *Pedicellina*. Although the rectum is highly ciliated, no revolving motion of the faeces has been observed in this species: in the rectum of *L. claviforme* and *L. singulare*, however, a very slow motion of waste matter has been noticed; that in the intestine revolves rapidly.

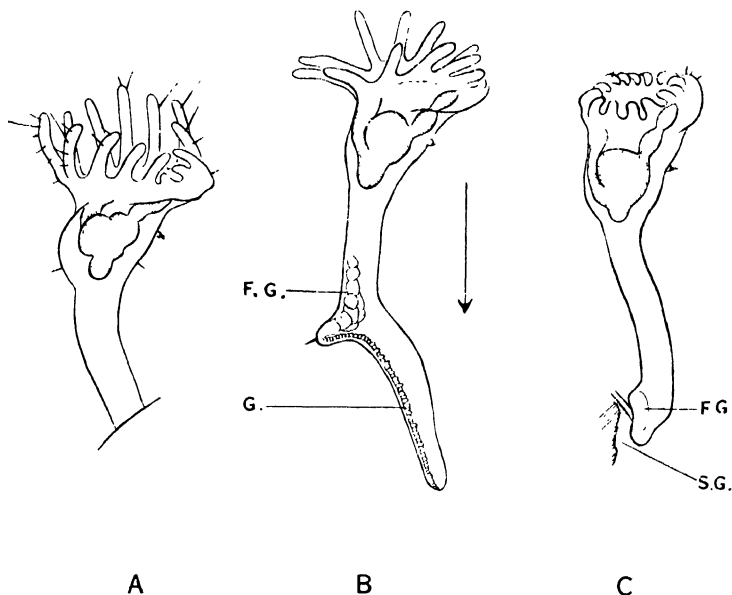
When a slight trace of neutral red is added to the sea-water practically the whole of the cells lining the alimentary canal take the stain, but very slowly, some granules in the rectal cells showing it first. The 'liver'-cells are naturally yellow in colour, and the granules in these become orange with neutral red. The cells of the apex of the stomach contain granules which become almost black red; in the living animal these appear as minute, shining, colourless globules. After an hour or so in sea-water tinted with methylene blue and then removal to clear sea-water, the originally yellow-coloured 'liver'-cells may acquire a distinct green tint. Assheton (1, p. 133) found that the

'liver'-cells of *L. saltans* were hardly affected by methylene blue.

All specimens examined over a period of about eleven months were budding freely; the greatest number of buds seen was four on one side, of which the youngest was a mere ridge, and three on the other as in Text-fig. 1, A; the buds occur near the ventral and distal wall of the stomach (see Text-fig. 1, A and B). They would appear generally to detach themselves from the parent, by violent contortions, bending the 'heel' to the tip of the foot, and the lophophore to the 'heel', when they have attained a length of about 0.65 mm., and possess fourteen tentacles of which four are more or less rudimentary. The two youngest tentacles, which appear as tiny rounded protuberances, occur on either side of the median plane at the distal edge of the lophophore (Text-fig. 2, A). New tentacles are therefore formed either in pairs or alternately—probably alternately in older individuals—in this position, the tentacles increasing in length ventralwards; one occurs on either side of the mouth. It is in the distal median line of the lophophore that the ciliated vestibular groove at the base of the tentacles is interrupted, and particles passing down the tentacles on either side of this point travel in opposite directions round the groove to the mouth. A rudimentary tentacle described by Salensky (29, p. 5, and Pl. 12, fig. 1) as occurring near the mouth has not been observed, but all tentacles are not fully extended at the same time. There is, however, considerable variation in length of the tentacles of different adult individuals of approximately the same size, the tentacles generally being shorter in old-looking individuals, with numerous large yellow granular excretory cells (see p. 327), than in transparent ones, with few of these cells. This is variation in length, and not in the state of contraction of the tentacles, as is evident from the difference in the number of groups of lateral cilia, and therefore of lateral cells of the tentacles, in the different specimens. There is a short row of about twelve to fifteen long cilia on each lateral cell, separated from those on adjacent lateral cells by a slight gap, corresponding to the cell-wall.

In the buds the stalk, together with the foot, is slender, and

when fully expanded is very graceful (Text-fig. 2, B). Free buds swim slowly; they move with the calyx hindmost as noted for the free buds of *L. nitschei* by Roper (28, p. 56).



TEXT-FIG. 2.

L. crassicauda. Living buds and young fixed form. $\times ca. 76.3$.

A. Bud attached to parent, showing addition of tentacles in the distal region of the lophophore. B. Bud sketched just after liberation from parent. C. The same individual as in B, sketched six days later, showing great reduction in length of the foot, and increase in length of the stalk. It is partly attached to a sponge spicule, and partly to a fragment of vegetable debris. It was already fixed three days after liberation, when it was in much the same condition as in the sketch. F.G., foot-gland; G., groove of foot-gland; S.G., secretion of foot-gland. The arrow indicates the direction of movement of the free bud in swimming.

Buds seem to become attached first—but slightly, for they are easily dislodged—by the extreme tip of the foot. The bud then appears to examine with the pointed heel, which bears one or two tactile hairs, the object it has touched; the ‘sole’ of the foot meanwhile being well arched. The foot may then

contract strongly, the whole 'sole' of the foot with its open 'duct' or groove being applied to the object. The contracted foot would then seem to be gradually reduced in size; in young individuals the termination of the stalk is more or less cylindrical (Text-fig. 2, c), or, as Schmidt (32, p. 72) has described it, like the foot of an elephant.

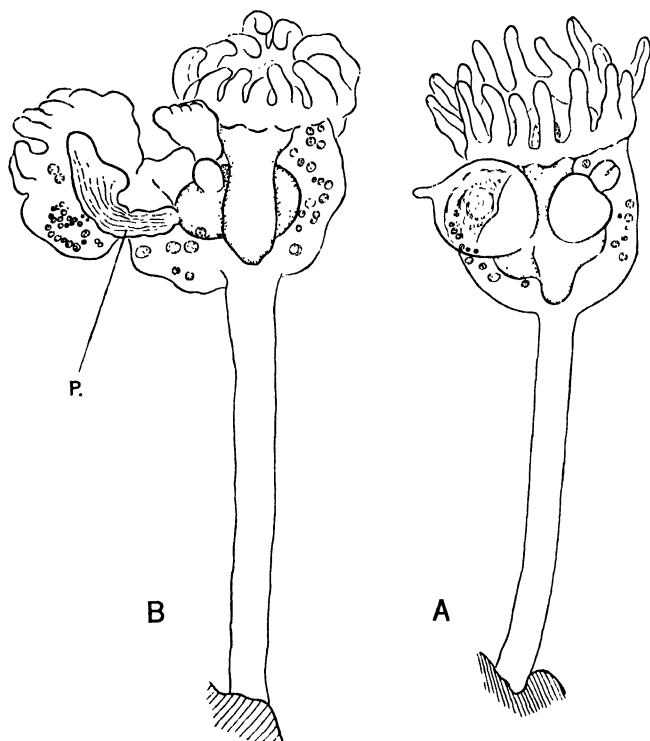
Abnormal Buds.

A few individuals with abnormal buds have been seen. In two or three instances the bud, though of considerable size, was a round mass of undifferentiated cells with a narrow projection at the summit. In the specimen figured (Text-fig. 3, A), however, there appeared to be an extension of the stomach of the parent into the bud of one side. In two specimens the abnormal buds consisted of little more than the lophophore, the vestibule being a slight depression, and without any digestive system. Attached to the parent near the base of one bud was a separate muscular process, which evidently represented the stalk and foot. In one specimen (Text-fig. 3, B) the lophophore of the abnormal bud faced in the opposite direction to that of the parent, and the two halves of the lophophore were unequally developed. This individual also bore normal buds, and a perfectly developed one, on the same side as the abnormal bud, freed itself just before the sketch was made. The second individual bore similar abnormal buds, one on either side, but with the lophophores facing in the same direction as that of the parent. A number of *Loxosomas* from the small tank in the Aquarium carried well-developed buds, which were slightly abnormal in that the foot was much shorter than usual, and although the groove or 'duct' of the gland was present, the gland itself appeared to be small or absent. These buds grew to a large size while still attached to the parent; apparently owing to the shortness, or lack, of the foot they experienced difficulty in freeing themselves.

Notes on the Life-history.

Batches of individuals, including young and adult forms, from different parts of the south wall of a shallow tank in the

Laboratory, were examined from March 1929 to February 1930. Throughout this time all those in which the sex was determinable were males; in the great majority of these the gonad was tiny,



TEXT-FIG. 3.

L. crassicauda. Living individuals with abnormal buds. $\times 57.25$. A. Ventral view of parent with large round abnormal bud. B. Ventral view of parent, showing dorsal surface of abnormal bud. Two small buds are present on the same side as the abnormal bud. P., muscular process attached near the base of the abnormal bud. Numerous cells, possibly having an excretory function, are present in the calyx.

composed of a small number of clear cells, and the presence or absence of a vesicula seminalis was relied on for the determination of sex. In only a few individuals was sperm seen, and when

present it was generally in one-half of the gonad only, that half being considerably larger than the other. The examination was made for the most part on specimens fixed in formalin, stained with borax carmine, and mounted in alcoholic canada balsam (see Harmer, 'Quart. Journ. Mier. Sci.', vol. 46, p. 264, 1902), and in some instances it was impossible owing to the position of the animals to determine whether or no a vesicula seminalis was present, so that the percentage of males is probably considerably higher than recorded in Table 1 (p. 335). In the living animal it was found almost impossible to be certain of the presence or absence of an empty vesicula seminalis, owing to the prevalence of waste particles in the intestine and rectum. No ova were seen in any of the specimens examined.

These results were so unexpected that a number of individuals from the small tank in the Aquarium were examined on August 19, 1929, for comparison, with the following result: out of nineteen individuals twelve at least were male, that is, about 63 per cent., while the sex of the remainder could not be ascertained; but here again the sex was determined by the presence of a vesicula seminalis, which in all cases was empty. The gonad in all specimens was tiny and no ova were seen.

A possible explanation of these results is that owing perhaps to lack of sufficient food in the unnatural conditions under which the animals were living, the development of the reproductive elements was very slow; the majority of those examined, however, were budding freely, though budding in *Loxosoma* does not normally seem to retard the development of the gonad, as in other species buds may be found on individuals with well-developed gonad, and even with the vestibule crowded with embryos. The fact that all individuals examined, including those up to 1.87 mm. in length, in which sex was determinable were male, would seem to point to the conclusion that in *L. crassicauda* individuals are first male; it would hardly seem to be probable that both tanks were colonized by male individuals of a dioecious species. The *Loxosomas* in both tanks must have been multiplying by budding alone.

It might be noted that the larva of *L. crassicauda* is at present unknown (see 9, p. 263).

TABLE I.
Loxosoma crassicauda: Proportion of Recognizable Males in Collections made from a Shallow Tank in the
 Laboratory during 1929-30.

Date.	Mar. 23, 1929.	May 2, 1929.	May 14, 1929.	May 28, 1929.	June 12, 1929.	June 26, 1929.	July 24, 1929.	Aug. 23, 1929.	Sept. 18, 1929.	Oct. 29, 1929.	Dec. 2, 1929.	Feb. 24, 1930.
Males:												
Number	4	17 (2 with sperm)	8 (2 with sperm)	15 (1 with sperm)	20	15	16	33 (9 with sperm)	0	17 (6 with sperm)	26	10
Per cent.	40.0	54.8	34.8	75.0	83.3	68.2	61.5	100.0	0	60.7	74.3	32.
Sex indeterminate:												
Number	6	14	15	5	4	7	10	0	4	11	9	21
Per cent.	60.0	45.2	65.2	25.0	16.7	31.8	38.5	0	100.0	39.3	25.7	67.
Totals	10	31	23	20	24	22	26	33	4	28	35	31

In order to obtain some idea of the rate at which individuals multiplied by budding, two free buds were isolated in finger-bowls. One produced twelve individuals in 85 days; its first bud became free when the parent was 43 days old and 0.9 mm. long. The other produced six individuals in 77 days; its first bud freed itself when the parent was 44 days old and 0.92 mm. long. In the latter instance the first two or three individuals were much worried by masses of bacteria, which enveloped the stalk and part of the body, and had to be removed at intervals. The seawater in the bowls was changed every few days, but no food was given. The results are therefore unquestionably much lower than they would be under natural conditions.

LOXOSOMA SINGULARE Keferstein (Text-figs. 4, 5, and 24, c).

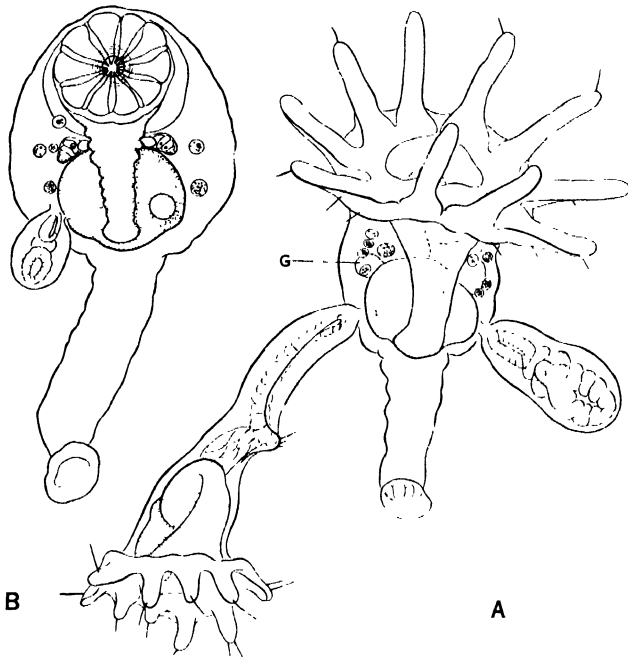
Notes on the Morphology.

A small species of *Loxosoma* found on the ventral surface of *Aphrodite aculeata*, and also on the dorsal surface and on the under side of the elytra, is most probably *L. singulare* (Text-figs. 4-5). In the latter position the *Loxosomas* lie more or less parallel with the elytron - the stalk near the base being bent almost at right angles - generally with the lophophores facing the elytron, but occasionally facing downwards into the 'respiratory chamber' of the *Aphrodite*.

This small species on *Aphrodite* has been identified by Barrois (**3**, p. 9), Hincks (**13**, p. 573), and Harmer (**9**, p. 262), as *L. singulare*, but there is some doubt as to its identification owing to uncertainty as to the presence, or absence, of a foot-gland in the bud of this species; one is certainly not shown in Claparède's figure (**4**, Pl. 11, fig. 6). Except for the presence of a foot-gland in the bud, the small species found on *Aphrodite* at Plymouth (Text-fig. 4) agrees in general with the description and beautiful illustrations of that author, its original discoverer, of a species he found on *Notomastus* (*Capitella*) *rubicundus* at St. Waast, Normandy, and which was named *L. singulare* by Keferstein (**16**, p. 181).

As *L. singulare* was the first species discovered and

described, it is possible that the presence of a foot-gland in the bud was overlooked; this seems probable as the anatomy of the animal was imperfectly understood; the anal extremity of



TEXT-FIG. 4.

L. singulare. $\times ca. 115.6$. A. Ventral view of living female individual with lophophore open. The gonad (G) is immature; the ovum on the left side is fairly large, though not opaque. Several large cells, possibly excretory in function, are present on either side of the oesophagus. In this individual the stalk did not end in a disc of attachment, and a vestige of the foot-gland was present. Sense-hairs are present on the 'heel' of the foot of the mature bud. B. Ventral view of mounted female individual with closed lophophore, showing the development of slight wings due to the contraction of the muscles of the calyx. The oesophagus shows ridges of contraction. The gonad contains immature ova in 'pagoda' arrangement. The stalk ends in a disc of attachment.

the intestine was thought to pierce the wall of the pharynx and open outwards in the middle of the mouth, and Claparède

(5, p. 29) was doubtful whether the part called by Keferstein (16) and himself (4) the mouth, might be the anus and vice versa.

Barrois (3) in 1877 described the embryology of a *Loxosoma* from *Aphrodite* which he identified as *L. singulare*. His specimens evidently had a foot-gland in the bud (see 3, p. 10 and Pl. 16, fig. 5), though not in the adult (p. 9). He found this species at St. Waast; it has since been doubtfully identified on the same host by Harmer (9, p. 262) at Naples.

Schultz (33, pp. 56, 57) in 1895, in his tabulated description of *Loxosoma*, has for *L. singulare* under the heading 'Drüse', 0; and then under 'Sonstige Merkmale' 'Der Fuss endigt mit einer Drüse', which is contradictory, unless the latter remark was intended to refer to the buds.

Efforts at Plymouth to obtain this species on its original host, in order to decide the question of identification, have been unsuccessful, the *Notomastus* dredged from the Rame mud—where *Aphrodite* carrying this species are taken—being free of the commensals.

L. singulare is described by Claparède (4, p. 106) and Keferstein (16, p. 131) as having 10 tentacles: Harmer (9, p. 262) was somewhat doubtful of his identification of the specimens at Naples, as in the very few he obtained the number appeared to be 12 or 13. Those on *Aphrodite* at Plymouth, though generally having 10 tentacles may have occasionally 8, 9, 11, or 12, and very rarely 13, depending roughly on the size or age of the individuals. It would appear that in *L. singulare*, as in perhaps the majority of the *Loxosomatidae*, the number of tentacles present in the bud on liberation undergoes a slight progressive increase with age; there are very few known species with a constant number of tentacles.

L. singulare is a small species. Claparède gives the length as 0.3 to 0.4 mm.,¹ and Keferstein as 0.4 mm. *L. singulare* at Plymouth varied between 0.18 and 0.8 mm. in length. Of 72 specimens measured, the average total length was 0.530 mm., with length of calyx 0.305 mm., and of stalk 0.225 mm. The

¹ In (4) Claparède gave the length as 3 to 4 mm., but later (5) corrected it to 0.3 to 0.4 mm.

average calyx width of 54 specimens was 0.179 mm. The width of the stalk varied between 0.03 and 0.08 mm.

The stalk is generally shorter than the calyx, though it varies somewhat in different individuals (an individual 0.7 mm. total length had an exceptionally long stalk 0.45 mm. in length), and ends generally in a disc of attachment (Text-fig. 4, B) (see also Hincks, **13**, vol. i, p. 573; and Harmer, **9**, p. 262), though in some specimens (Text-fig. 4, A) it appears to be absent. Claparède's (**4**, p. 106) description of the stalk and disc is as follows: 'Der Stiel ist farblos und breitet sich in eine rundliche Haftscheibe, durch deren Hülfe der Schmarotzer auf Capitella (Notomastus) festsitzt, aus. Nimmt man die Unterseite der Fuss Scheibe in Augenschein, so findet man, dass die ganze Sohle mit zahlreichen, 0.014 mm. breiten rundlichen Zellenkernen besetzt ist, die unmittelbar unter der farblosen Cuticula sitzen.' He, however, does not say that the animal was capable of changing its position (as can *L. davenporti*, **23**, p. 355; and *L. saltans*, **1**, p. 124), nor does Keferstein (**16**). Keferstein (**16**, p. 131) describes the stalk 'mit dessen fussartiger Ausbreitung er sich auf der äusseren Haut der Annelide befestigt'. In the Plymouth specimens once the animals are attached they do not change their position; they would appear to be attached by a secretion poured out by the foot-gland before this atrophies. In many adults a vestige of the foot-gland is present. Specimens on the ventral surface of *Aphrodite* are often attached by their discs to the brown-coloured papillae of the worm; when the surface is scraped the latter are broken off at their fine necks, giving to the *Loxosoma* from this position the appearance of having a very large brown disc of attachment. Longitudinal muscles only are present in the stalk.

The buds occur near the ventral and proximal wall of the stomach, the greatest number seen so far being two on each side. Keferstein apparently saw only one on either side (**16**, p. 132), and Harmer says of his specimens that none possessed more than a single bud, which was provided with a foot-gland, while Hincks (**13**, vol. i, p. 573) found as many as three buds present on a side, on specimens from *Lactmonice*

filicornis from Shetland. Probably the number of buds present varies, perhaps with the season or with the food supply. Buds may be present on sexually mature individuals of either sex. Budding individuals have been observed in March, April, June, July, September, and October.

True nephridia as described by Harmer for *L. crassicauda* are present; in addition, large yellowish granular cells, with a possible excretory function, are present in the calyx on either side of the stomach (see also p. 327).

The generative organs are paired, and, so far as is known, the sexes are separate. The number of recognizable ova present in the gonad is small; the ripe ovum is large (*ca.* 0.08–0.09 mm. in diameter) and yolky. A paired shell-gland occurs in connexion with the oviduct (see also Prouho, **27**, p. 105). In females carrying a number of embryos the vestibule is produced into two lobes, one on either side of the rectum (Text-fig. 5, A–B). Harmer (**9**, p. 285) states that *Loxocalyx* (*Loxosoma*) *leptoclini* possesses ‘two specialized diverticula of the posterior portion of the vestibule, one on either side of the intestine, which by its projection as a large longitudinal ridge (covered, of course, by ectoderm) into the vestibular cavity gives rise to the two diverticula’: in this species, however, the epithelium lining the diverticula is modified for a nutritive purpose. As many as nine or ten embryos may be present simultaneously in the vestibule in *L. singulare*.

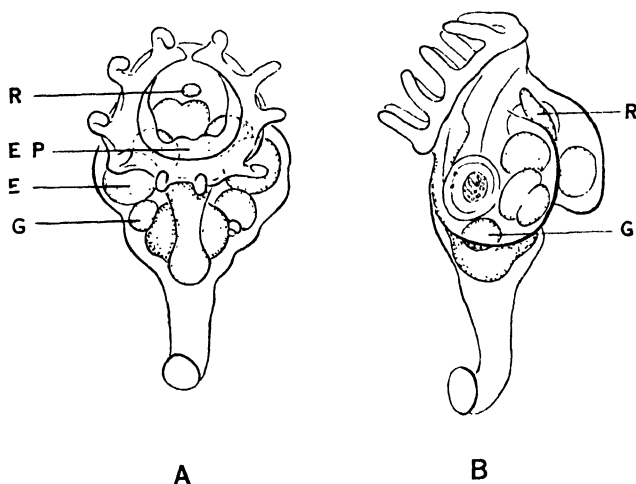
The ripe male gonad is larger than the mature ovary.

The development of the larva has been described by Barrois (**3**, pp. 10–54). It might be noted that the anal cone of the larva of *L. singulare* bears long cilia, and in addition on either side a stiff tactile hair, although Barrois figures it as being destitute of cilia (**3**, Pl. i, fig. 21). While the eye spots may be ‘une couleur carmin’ as described by him, in some larvae they have been observed to be a reddish brown. Barrois found embryos in the vestibule during July at St. Waast: at Plymouth they have been seen in the vestibule from April to October; as few specimens have been obtained during the winter months it is impossible to say that breeding does not occur during those months.

Little information has been gathered as to the proportion of the sexes, as this species, though found on a good proportion of *Aphrodite*, was generally present in very few numbers.

The only records are as follows:

September 1930. (1) On the dorsal surface of an *Aphrodite*



TEXT-FIG. 5.

L. singulare, sketches of living individuals with embryos in the vestibule. A. Ventral view, lophophore widely open. Five embryos are visible in the vestibule. B. Side view, showing the vestibule produced into two lobes, one on either side of the rectum. Two embryos (unstippled) are almost ready for liberation. *E*, embryo; *EP*, epistome; *G*, gonad; *R*, rectum. $\times ca. 85-9$.

10 *L. singulare* were obtained, of which 9 were female, and 1 with the gonad too tiny for the sex to be determined. This group, judging from the large bilobed vestibule, with at most one or two embryos (in one instance only an empty envelope), and the small gonad, was probably at the end of a reproductive phase.

On the ventral surface of the same 'host' 2 males only were taken; they were fully mature, the gonad containing sperm.

The females varied between 0.42 and 0.6 mm. in length; the 2 males were 0.38 and 0.53 mm.

(2) On the same date, on the dorsal surface of another *Aphrodite*, out of the 7 individuals obtained, 5 were female, and 2 had the gonad too small for the sex to be determined. The females had one or two large opaque ova in the gonad, and a well-developed embryo in the vestibule. The females were between 0.44 and 0.64 mm. in length; the individual with indeterminable gonad was 0.40 mm.

October 1930. From the dorsal surface of an *Aphrodite* 27 *L. singulare* were taken of which 20 were female, 1 male (with sperm in gonad), and 6 with gonad too small for sex to be determined. Of the females, 13 had a single embryo in the vestibule, 8 in early and 5 in late stages of development, and 1 female had an opaque ovum in the gonad; the remainder had small ova. The females were between 0.42 and 0.8 mm. in length; the male was 0.52 mm. long; those of which the sex could not be determined were between 0.43 mm. and 0.7 mm. The probable explanation of indeterminate gonad in large specimens is that the gonad was spent.

Distribution in the Plymouth Area.

The *Aphrodite* examined have come chiefly from the Looe and Rame Eddystone Grounds, and in fewer numbers from the Mewstone Grounds; a single individual carrying a considerable number of *L. singulare* came from Bigbury Bay. *L. singulare* was present on 56 of the 141 (39.7 per cent.) *Aphrodite* examined between October 1927 and October 1930, though in most instances in very small numbers. They occur much more frequently on the ventral surface than on the dorsal, but in the latter position usually occur in much greater numbers. When on the dorsal surface they are not restricted in position as is *L. obesum* (see p. 355), and may occur on the anterior elytra.

Double Specimen.

A double specimen of *L. singulare* (Text-fig. 24, c, p. 386) was observed in March 1930. It had a common stalk and the bodies united side by side; the two lophophores were

distinct. The oesophagus and rectum of the two individuals were separate, but there was a single bilobed stomach. The specimen was not in good condition when found, and it was impossible to distinguish the nerve ganglion or gonad. No buds were present. This specimen is similar to the third condition of union described by Nickerson (22) for double specimens of *L. davenporti*, except that the single stalk is much broader than normal.

LOXOSOMA CLAVIFORME Hincks (Text-figs. 6-7).

L. claviforme was discovered by Hincks (13, vol. i, p. 575) on *Hermione hystrix* from shallow water, Guernsey. He was somewhat doubtful of the validity of the species as his material had been long preserved in alcohol, and he admits that his diagnosis is very incomplete. The characteristics he gives are as follows: 'Body ovate; tentacles (probably) 10 or 12; peduncle somewhat longer than the body, tapering off gradually downwards, and terminating below in a short, foot-like expansion—the whole figure very regularly clavate when the tentacles are withdrawn; ? pedal-gland; only a single bud observed, placed about half-way down the body.'

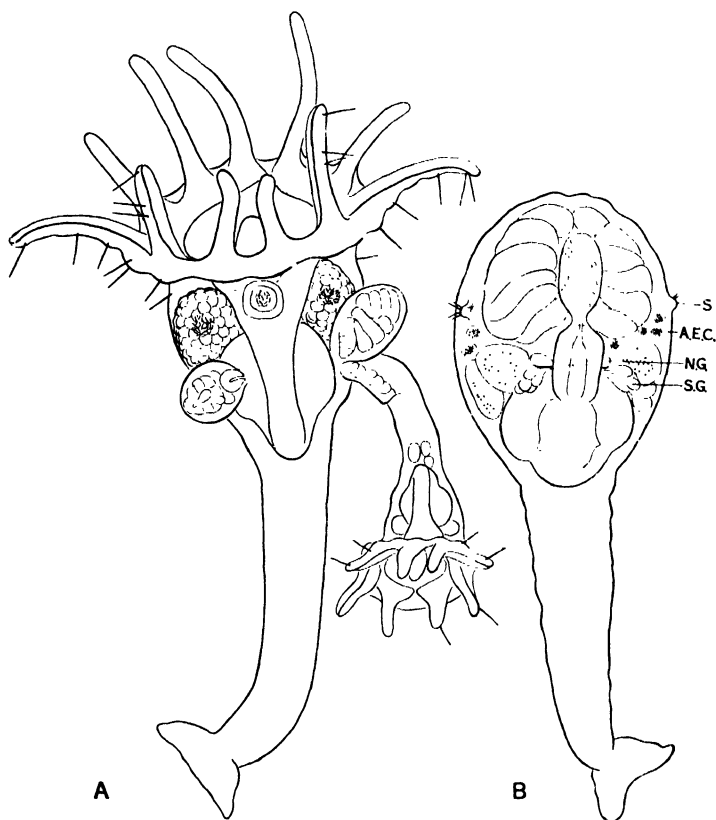
L. claviforme (Text-figs. 6-7) very closely resembles *L. singulare* (Text-figs. 4 and 5, pp. 337, 341) from *Aphrodite aculeata*, and Harmer thought it better provisionally to identify with this latter species the few specimens of *Loxosoma* he found on *Hermione hystrix* at Naples, until the distinctness of *L. claviforme* could be more satisfactorily shown (9, p. 263). He suggested to me that a renewed examination of these species was desirable, and with this object any specimens of *Hermione* brought in by S.S. 'Salpa' have been examined. Very few individuals have been obtained so far: two only carried *Loxosoma*, and one of these had been crushed so badly that the few commensals present were useless for identification purposes; the other had several dozen specimens in perfect condition, and from these it has been possible to work out the characteristics of the species. It is regrettable, however, that more 'colonies' were not obtained, as it is possible there may be some variation in form in different 'colonies'.

Habitat.

The *Loxosoma* occurred chiefly on the parapodia (Text-fig. 7, A, p. 348), being more numerous anteriorly and posteriorly, while a few were found on the ventral surface. In preserved specimens of *Hermione* from the Museum at the Laboratory, not only were they found on and between the feet, and practically all over the ventral surface, but also on the dorsal surface, where they were more abundant on the body-wall, beneath the elytra, than on the under-surface of the elytra themselves.

Description.

As previously mentioned, *L. claviforme* (Text-figs. 6-7) much resembles *L. singulare*, but differences in size, number of tentacles, position of the budding zone, and above all the presence of paired sense-organs in *L. claviforme*, clearly distinguish the two species. I have been unable to find sense-organs in *L. singulare*, though one or two stiff sense-hairs are present in this species in a similar position. The probable evolution of a sense-organ from a sense-cell (see Harmer, 9, p. 274), makes the distinction between the two difficult when sense-organs are little developed. The sense-organs of *L. claviforme* are small papillae, bearing a few (3 to 6) stiff tactile hairs, situated one on either side of the calyx (Text-fig. 6, B), but as in *L. crassicauda* and *L. phascolosomatum* they are somewhat dorsal in position, and therefore difficult to see unless the animal is in a certain position. The vestibule, in individuals with embryos, becomes produced into two lobes or pouches, one on either side of the rectum, as in *L. singulare* (see p. 340) and *L. leptoclini* (9), and the sense-organs are on the outer side of these. Possibly their position has some reference to the safeguarding of the embryos while in the vestibule. They are roughly in the same position as those of *L. phascolosomatum*, but are slightly more distal than those of *L. crassicauda*, and are not so well developed. It has not been noticed that they are retractile, as are those of *L. phascolosomatum*. *L. claviforme*



TEXT-FIG. 6.

L. claviforme. A. Ventral view of living male individual with lophophore open. A small amount of sperm is present in the gonad of either side, and in the vesicula seminalis. The large bud is slightly abnormal in that the foot is short, and a foot-gland is absent, though the groove of the gland is present. B. Dorsal view of living unnarcotized female individual with closed lophophore to show the general clavate shape and the paired sense-organs (*S.*). Small clear globules are present in the ova. Yellow globules and fine granules are present in the cells of the side-walls of the rectum. *A.E.C.*, large yellow granular cells; *N.G.*, nerve ganglion; *S.*, sense-organ; *S.G.*, shell-gland. $\times ca. 115\cdot6$.

would seem to resemble *Loxosomella antedonis* Mort. (19), both in the general shape and size, and the position of the

budding zone, but the sense-organs of that species appear to be of a different type.

Stiff tactile hairs are present on the unciliated surfaces of the tentacles and scattered over the calyx (Text-fig. 6, A), as they are perhaps in all, or most, species of *Loxosomatidae*.

L. claviforme apparently attains a larger size than *L. singulare*, though size is perhaps rarely a reliable distinguishing character unless numerous specimens have been observed at different times. Living expanded individuals varied between 0.6 mm. and 1.0 mm. in length. Of 23 specimens measured, the average total length was 0.80 mm., with length of calyx 0.37 mm., and of stalk 0.43 mm. The average width of calyx of 22 specimens was 0.23 mm.: the stalk varied between 0.07 and 0.09 mm. in width. As the smallest specimen measured was 0.6 mm. in total length, while buds almost ready for liberation were about 0.32 mm. to 0.34 mm., it is probable that the averages are rather higher than they should be for comparison with those of *L. singulare* (see p. 338), in which the smallest specimen measured was only 0.18 mm. long.

In *L. claviforme* the stalk, on the whole, is longer in proportion to the body than in *L. singulare*; it is rarely shorter than the body, as is usual in *L. singulare*.

This species was named for its general clavate shape with closed lophophore (see **13**, vol. ii, Pl. 81, figs. 9 a and 10; and also Text-fig. 6, B, of this paper): *L. singulare*, however, with closed lophophore is sometimes of this shape.

The number of tentacles in the single 'colony' in which counts of these could be made was generally 12; actual figures are as follows: out of 23 specimens, 2 had 13 tentacles, 14 had 12 tentacles, 6 had 11 tentacles, and 1 had 10 tentacles. In *L. singulare*, on the other hand, the common number is 10; for example, out of 65 individuals, 3 had 11 tentacles, 47 had 10 tentacles, and 15 had 9 tentacles. Specimens of *L. singulare* with 12 and even with 13 tentacles have been seen, but are rare; there seems a distinct tendency in *L. singulare* to have a smaller number of tentacles than *L. claviforme*. Mature attached buds of *L. claviforme* 0.32 mm. and 0.34 mm. long had 8 tentacles.

Hincks says that the stalk ends in a 'short, foot-like expansion'; in the specimens on *Hermione* at Plymouth, however, the stalk ended in a small disc of attachment, sometimes perfect in shape, sometimes irregular—this may be Hincks's foot-like expansion—the form possibly depending on the contour of the surface of attachment. The disc, as in *L. singulare*, is fixed to the 'host' by a substance secreted by the foot-gland before it atrophies, and is not a sucking disc.

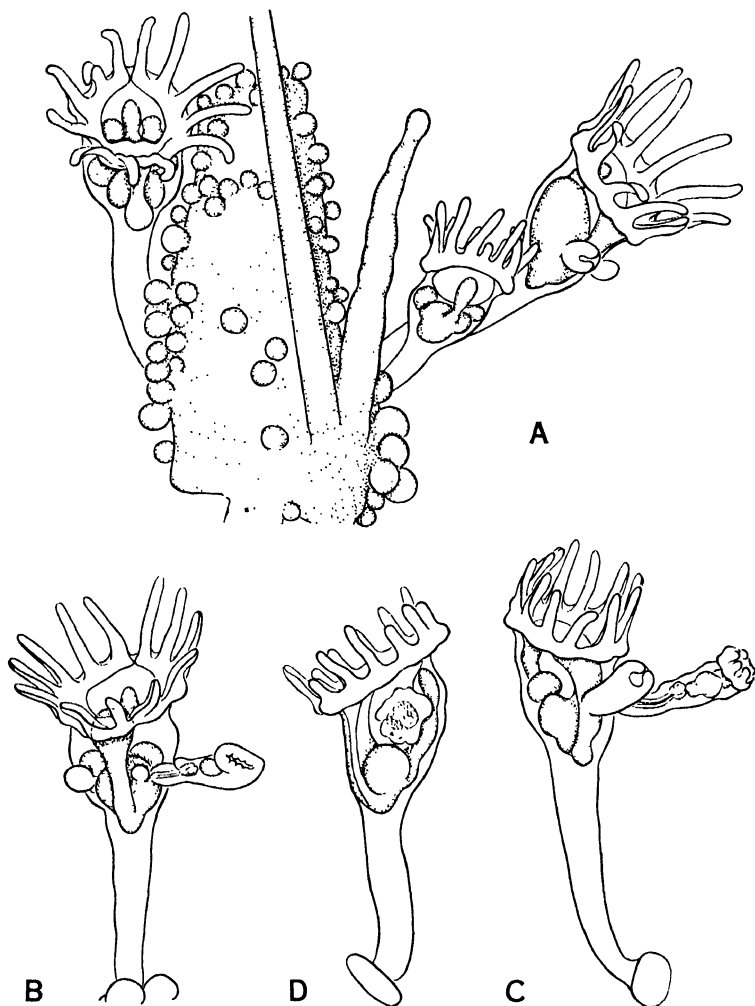
The stalk has longitudinal muscles only.

The budding zone (see Text-figs. 6, A; 7, B), as indicated in Hincks's fig. 9, Pl. 81, is rather more distal than in *L. singulare*, being in the region of the distal and ventral wall of the stomach. The greatest number of buds seen was two on each side; they are provided with a large foot-gland and groove. A few specimens were seen carrying slightly abnormal buds; the abnormality consisted in shortness of the foot, and absence of a foot-gland, though the groove of the gland was present (see Text-fig. 6, A).

The alimentary canal and generative organs of *L. claviforme* are similar to those of *L. singulare*. The cells of the side-walls of the rectum contain large yellow spherules and fine granules. The shell-gland of the female is large (Text-fig. 6, B). In the male the mature gonad is large, and the thick-walled vesicula seminalis is conspicuous (Text-fig. 6, A); a small amount of sperm was seen rotating in the vesicula seminalis of one individual.

A certain number of specimens in August and September carried embryos in the vestibule, the largest number seen being 6; it is possible, however, that the maximum number may be greater. No free larvae were seen.

Of 23 individuals examined for sex, 19 were female (13 with one or more embryos in the vestibule), 2 were male (1 with sperm, the other with a large gonad, though no sperm was visible), and 2 with the gonad too small for the sex to be determined. The females were between 0.64 and 1.0 mm. in length; the 2 males 0.6 mm. and 0.78 mm. long; and the 2 individuals in which sex could not be determined 0.9 mm. and 1.0 mm.



TEXT-FIG. 7.

L. claviforme. Sketches from life. $\times 57.25$. A. Parapodium of *Hermione hystrix* with attached *L. claviforme*. Two of the individuals are females, one with embryos in the vestibule, and a large opaque ovum in the right half of the gonad. B. Ventral view of female. One embryo is present in the vestibule. The end of the stalk is surrounded by papillae of the host. C. Side view of female, with buds. D. Side view. One well-developed embryo, showing eye-spots, is present in the vestibule.

True nephridia are present on either side of the oesophagus. As in the other species described in this paper (see pp. 327, 340, 370), some (about six) large granular cells, yellowish in colour, are present on either side of the oesophagus (Text-fig. 6, B). The granules contained in these cells become dark red with neutral red intra-vitam staining.

L. claviforme may be distinguished from *L. singulare* by: (1) its greater size, with stalk generally longer than the calyx; (2) greater number of tentacles; (3) more distal position of budding zone; and in particular (4) the presence of paired sense-organs.

LOXOSOMA sp. WITH ABNORMAL, SEXUALLY MATURE BUDS
(Text-figs. 8-9).

Description.

A small group of about sixteen interesting *Loxosomas* was found on the anterior clytra and body-wall of a small *Aphrodite aculeata* about 9 cm. long, from the Mewstone Grounds in October 1930. While the habitat was that of *L. singulare*, the specimens appeared to be intermediate between that species and *L. claviforme* (see Text-fig. 8).

They agreed with *L. singulare* in:

1. The number of tentacles. Of 14 specimens, apart from abnormal buds, 2 had 11 tentacles, 9 had 10 tentacles, 2 had 9 tentacles, and 1 had 8 tentacles.
2. The absence, or minuteness, of sense-organs.

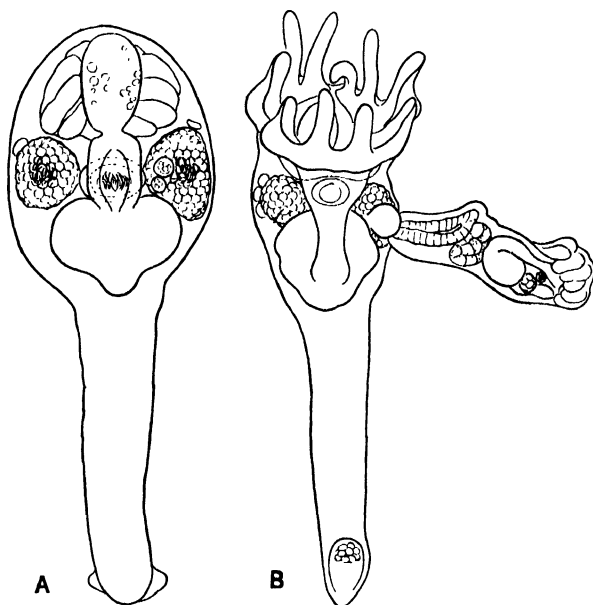
They agreed with *L. claviforme* in:

1. Size. The length of adults varied from 0.32 mm. to 1.075 mm.; the average length of 16 individuals being 0.69 mm., with length of calyx 0.33 mm., and of stalk 0.36 mm. The average width of the calyx of 12 individuals was 0.20 mm.; the width of the stalk varied between 0.05 and 0.09 mm.
2. The position of the budding zone in the majority of specimens (see Text-figs. 8, B, and 9, A).
3. The shape of the foot: in some individuals it was foot-like, as described by Hincks for *L. claviforme*.

The shape of the *Loxosoma* with closed lophophore

(Text-fig. 8, A) is that of *L. claviforme*, but possibly little importance attaches to this character, as *L. singulare* also may have this shape on occasion.

Most of the adults of any size were in a dirty condition,

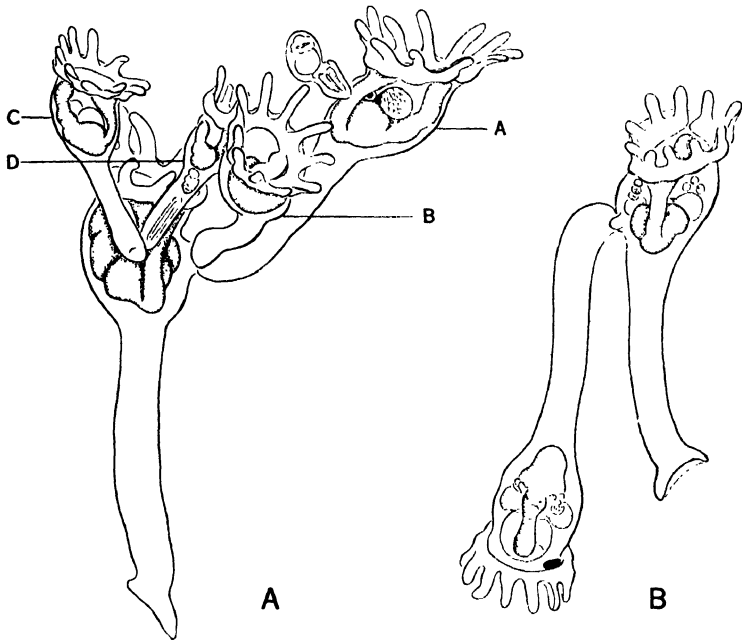


TEXT-FIG. 8.

Loxosoma sp. Living male individuals. $\times ca. 115-6$. A. Dorsal view of solitary adult with closed lophophore, to show the clavate shape. The large gonad contains sperm in both sides; also a small amount of sperm is present in the vesicula seminalis. On the right side two large cells, containing granules, lie dorsal to the testis. This individual had ten tentacles. Specimen unnarcotized. B. Ventral view of an abnormal 'bud', which became separated from the parent on lifting it. A few large cells at the base of the stalk are perhaps a vestige of a foot-gland. The gonad was composed of many small clear cells, but no sperm was present. The large bud, carried by this 'bud', appeared to be normal. By the appearance of the budding zone on the right side a bud had not long freed itself.

and with numerous fine, colourless threads (bacteria?) attached to them. They had a granular appearance, and this, together with the particles adhering to them, made it difficult to see the inter-

nal organs. A peculiarity of the 'colony' was the number and size of the large granular yellowish cells (excretory cells?, see pp. 327, 340) present in the calyx on either side of the oeso-



TEXT-FIG. 9.

Loxosoma sp. Sketches of living individuals carrying abnormal buds. $\times 57.25$. A. The parent is female, and has ten tentacles; of the 'buds' (A) has eleven tentacles and is a male, with vesicula seminalis full of sperm; (B) has ten tentacles and is a male; (C) has ten tentacles and appeared to be a young female; and (D) has eight tentacles and the gonad was too small for the sex to be determined. B. Sketch of a parent carrying an abnormal bud as large as itself. The sex of both parent and 'bud' is female.

phagus; in some individuals as many as fourteen occurred on either side.

Although several specimens had large ova in the gonad, none carried embryos in the vestibule.

A most interesting peculiarity of six of the sixteen specimens ($37\frac{1}{2}$ per cent.) was the retention of the buds (Text-fig. 9), so

that in an extreme case the bud was as large as the parent (see Text-fig. 9, B), and in some instances itself bore good-sized buds, which might be normal (Text-fig. 8, B). The abnormality seemed to consist in the absence of a heel to the foot, though a foot-gland and canal were present in at least some buds, and the gland functioned judging by the debris collected round the point of attachment of the abnormal bud to the parent. In normal buds the muscular foot would appear to play an important part in the liberation of the bud from the parent, and the inability of these buds to free themselves, most probably, was due to the abnormality of the foot. As previously mentioned a few specimens of *L. claviforme* (see Text-fig. 6, A, p. 345) from *Hermione hystrix* bore abnormal buds in which the foot was short, and though the foot-gland itself was absent, its groove was present.

Sex of the Buds.

The continued attachment of the 'buds' to the parents, resulting in 'buds' with mature gonad, made it possible to obtain some information on the question of the sex of the bud. Vogt (36, p. 335) says of *L. phaseolosomatum* '... m'engage à penser que le sexe des bourgeons doit être celui des individus sur lesquels ils ont été produits'. From the few specimens with abnormal buds in this small colony it is evident that though the sex of the bud may be that of the parent, it is by no means always so. The sex, size, and number of tentacles (where these could be determined) of the six individuals and their abnormal buds is given in the table on p. 353.

From the fact that a parent may bear buds of different sex from itself, it seems very probable that in this form at least there may be a change of sex.

In this small colony there was little difference in the proportion of the sexes. Of the sixteen adults examined, eight were female, six were male, one had the gonad too tiny for sex to be determined, and one had one side of the gonad male, though the female shell-gland was present. Females were on the whole of larger size than the males, but the numbers are much too small for the results to be of value. It might be noted that the single

PARENT.			BUDS.		
<i>Sex.</i>	<i>Total length in mm.</i>	<i>No. of tentacles.</i>	<i>Sex.</i>	<i>Total length in mm.</i>	<i>No. of tentacles.</i>
(1) ♀	0.9	?	(a) ♂ (see Text-fig. 8, B)	0.6	10
—	—	—	(b) ♂?	0.64	10
(2) ♀ (see Text-fig. 9, A)	1.075	10	(a) ♂ (large gonad; vesicula seminalis full of sperm)	0.8	11
—	—	—	(b) ♂ (large testes)	0.55	10
—	—	—	(c) ♀ (young)	0.475	10
—	—	—	(d) sex indeterminate	0.35	8
(3) ♀	0.78	11	(a) ♂?	0.35	?
—	—	—	(b) too tiny for sex to be determined	0.36	8
—	—	—	(c) too tiny for sex to be determined	0.34	8
(4) ♀ (fairly large ova)	0.65	10	(a) ♂	0.39	10
(5) ♂ (sperm in gonad)	0.65	?	(a) ♀ (recognizable ova)	0.46	10
(6) ♀ (see Text-fig. 9, B)	0.86	10	(a) ♀	0.86	10

specimen which seemed perhaps intermediate in sex was very near in size to the largest male and the smallest female. Sections of this individual confirmed the presence of a large shell-gland, and of sperm in the gonad of one side. The gonad of the other side could not be distinguished, and its place was occupied by very large granular excretory cells. No vesicula seminalis could be made out. It is perhaps possible that this individual was abnormal rather than intermediate in sex.

There is perhaps some slight possibility that the specimens forming this small 'colony' are hybrids between *L. singulare*

and *L. claviforme*. The number of individuals which have indefinitely retained their buds perhaps shows that there is something abnormal in their constitution, though abnormal buds of a similar type, though not sexually mature, have been found in *L. claviforme* (see p. 347), and among a colony of individuals (those in a small tank in the Aquarium) with all the characteristics of *L. crassicauda* (see p. 332).

There seems to be no reason why *L. claviforme* should not be found on *Aphrodite*, or *L. singulare* on *Hermione*.

LOXOSOMA OBESUM sp. nov. (Text-figs. 10-24).

Habitat and Distribution.

*L. obesum*¹ is found on *Aphrodite aculeata*; living specimens were first obtained during a visit to the Plymouth Laboratory in 1923. This species occurs on the dorsal surface of the worm and on the under-surface of the elytra; on two occasions only a single specimen has been found on the ventral surface, where *L. singulare* is most frequently taken. The *Loxosoma* is roughly restricted to the anterior half of each elytron, this being the only part which has a free surface to the space beneath; the posterior half overlaps the anterior half of the elytron behind, and the two surfaces come into close contact during rhythmical movement of the elytra. The *Loxosoma* inhabits a kind of respiratory chamber, for in *Aphrodite* the function of respiration is carried out by the thin dorsal body-wall, and a current of water is kept continually moving over it in an antero-posterior direction by the rhythmical movement of the elytra (7, 8). The water enters the 'chamber' by percolating through the felt which acts as a strainer, preventing the entry of fine mud; it is forced out of a posterior aperture by the depression of the elytra on to the dorsum, the movement beginning with the anterior pair and passing backwards (see Fordham, 8). The *Loxosoma* is therefore well situated in relation to a presumably food-bearing current. In *Aphrodite*, which have their respiratory surface very thickly

¹ I am indebted to Sir S. F. Harmer for suggesting the name.

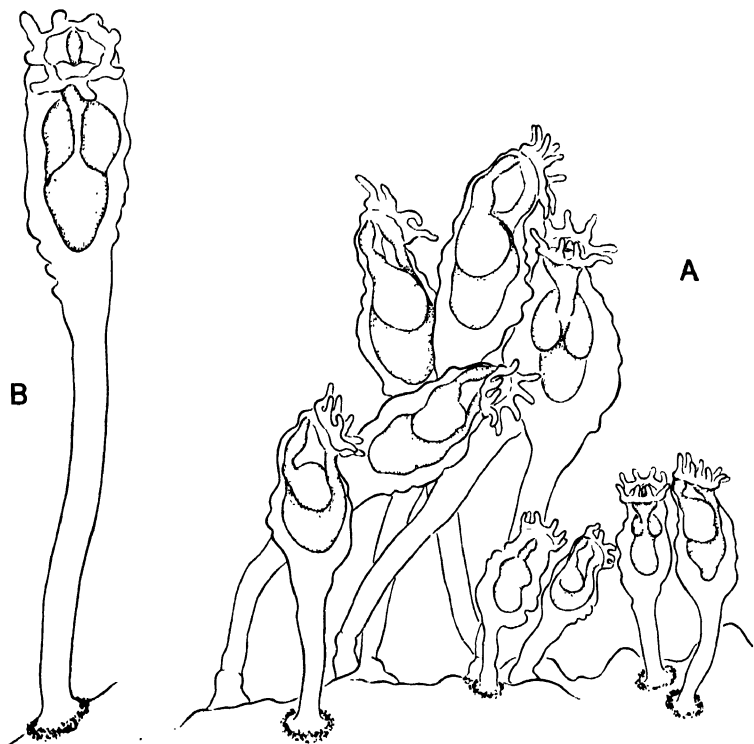
covered with large *Loxosomas*, it seems possible that they may interfere with respiration. Whether on the elytra, or on the dorsal surface of *Aphrodite*, the *Loxosoma* is practically confined to the posterior two-thirds of the worm; *L. singulare* is not thus restricted in position (see p. 342). In those worms in which great numbers were found, they were absent anteriorly to the seventh pair of elytra. This restriction in position doubtless has a close connexion with the conditions existing beneath the elytra; possibly the current in the anterior third of the 'chamber' is feeble compared with that in the more posterior part, but it is worth noting that it is from the anterior part of the worm that nephridia are absent. The genital products of *Aphrodite*, together with the coelomic fluid and its contents, pass by way of the nephridiopores into the current beneath the elytra, and may just possibly form an additional source of food for the *Loxosomas*.

While *L. singulare* has been found, though mostly in small numbers, on a fair percentage (39.7 per cent.) of *Aphrodite* examined, *L. obesum* is rather rare, though when the worm is infected, it is generally heavily. Out of a total of 146 *Aphrodite* examined between October 1927 and October 1930, only eighteen were infected, that is, 12.3 per cent. Infected worms have been obtained chiefly from the Looe and Rame Eddystone Grounds, though one carrying a few *L. obesum* came from the Outer Mewstone Grounds.

Description.

L. obesum is a large species; individuals may reach a length of 2.4 mm., while average individuals are rather more than 1.0 mm. in length. Several other large species of *Loxosoma* are known, but all differ from *L. obesum* in having a much larger number of tentacles; *L. davenporti* (23, pp. 352 and 374), 0.74–2.4 mm. in length, has 18 to 29 tentacles; *L. kerfersteini* (25, pp. 364, 365, 367), about 1.4 mm. long, has 14 tentacles; *L. crassicauda*, up to 1.87 mm. long, has 16 to 18 tentacles; *L. phascolosomatum* (36), about 1.8 mm. long (6, p. 59), has 12 to 18 tentacles; and *L. lanchesteri* (12, p. 5), up to 1.23 mm. long, has 20 or more tentacles.

In *L. obesum* (Text-figs. 10-13) the lophophore is small and circular, the small size being especially striking in large individuals. The number of tentacles is very constantly 8, but

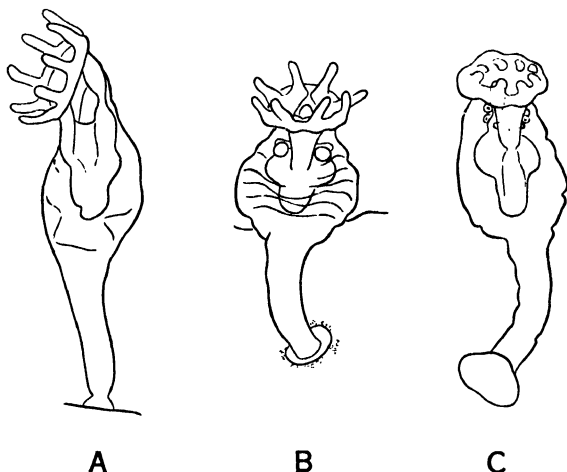


TEXT-FIG. 10.

L. obesum. Living individuals. A. A small group on an elytron of *Aphrodite aculeata*, showing the general appearance of the type with normal development of the stomach. The gonads were immature, and are not shown. $\times 41.4$. B. An individual of very elongated form from the same elytron as the group sketched in A. $\times 57.25$.

in one infection 2 out of 58 individuals examined had 9 tentacles, while later 1 was seen with 10 tentacles. *L. cochlear* Schmidt (31) and *L. pusillum* Harmer (12, Pl. I, figs. 19 and 20), 2 species which also have 8 tentacles, have a large

lophophore in proportion to the width of the body, as compared with that of *L. obesum*. The tentacles are short and small. In specimens in which the gonad is immature the calyx narrows considerably just below the lophophore (see Text-fig. 10); proximally it is generally clearly marked off from the stalk, which varies considerably in length (cf. Text-figs. 10 and 13, A),



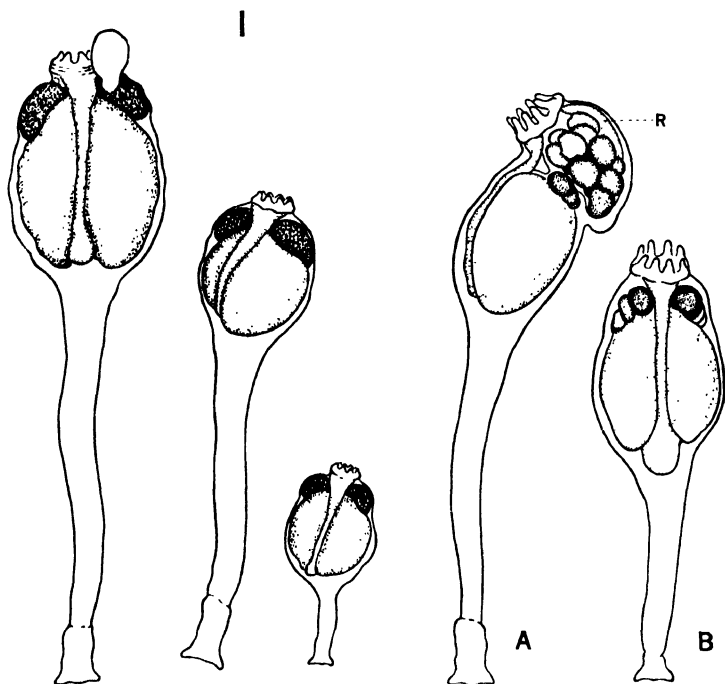
TEXT-FIG. 11.

L. obesum. Small individuals with normal development of the stomach. A. Side view of living specimen with lophophore well expanded and distinctly oblique in position. $\times 63$. B. Ventral view of specimen mounted unstained in glycerine, showing slight development of wings owing to the contraction of the muscles of the calyx. A tiny bud is present on either side. $\times 57.25$. C. Ventral view of living, immature male, with lophophore partly closed. Two large cells, with greenish yellow central granules, are present on either side of the oesophagus. $\times 63$.

and may be anything up to half to two-thirds of the total length. The stalk ends in a disc of attachment (not a sucking disc); in the larger specimens the cuticle of the lower part of the stalk may be thickened and brownish in colour (Text-fig. 12), and there is generally a collection of dirt particles round the base of the stalk. Longitudinal muscles only are present in the stalk.

In the buds a large foot-gland, with its groove, is present; this is preserved as a vestige only in many adults, while it is absent in others.

Variation in Shape of the Calyx.—*L. obesum*,



TEXT-FIG. 12.

L. obesum. Living individuals with large, swollen, oval-shaped stomachs. I. Three mature males to illustrate the variation in size of mature males. The two largest individuals had the end of the stalk thickened and brownish in colour. The almost terminal position of the tiny lophophore in mature males is well shown in these specimens. $\times 35.5$. II. Mature females. A has an enlarged vestibule containing many embryos. A. $\times 35.5$; B. $\times 49.1$. R., rectum.

with its marked variation in the shape of the calyx in different individuals, is a striking example of the difficulty of defining satisfactorily species of *Loxosoma* which have no specialized structures, such as sense-organs, cirriform or glandular organs.

In some instances (see Text-figs. 10-13) it is difficult to believe that the animals are of the same species, except for the constant characteristics, the number of tentacles and small size of the lophophore, and the habitat.

L. obesum occurs under two main forms or phases: (1) the one in which the stomach is normally developed for the family (Text-fig. 10); and (2) the other in which the stomach, owing to the exceptional development of the so-called liver-cells, is much enlarged, occupying by far the greater part of the calyx (see Text-figs. 12, 13).

(1) In individuals with the stomach normal in size, the calyx may vary between the broad form (Text-fig. 10, A) and the very narrow and elongated (Text-fig. 10, B), the shape, no doubt, depending to a large extent, if not wholly, on the degree of muscular contraction. (Both figures are of individuals narcotized with stovaine.) Text-fig. 10 A and B, are of individuals in the same 'colony' or infection, but the same animal was not observed actually to change from one form to the other. Text-fig. 11 A-C, are of small individuals of this type. The stomach varies somewhat in shape; it may be distinctly elongated with well-marked lateral lobes, and there may be a large apical region, or it may be almost round, the lateral lobes only indicated by the greater depth of their cells. This variation in shape is evidently due to the great contractility of the animal. The 'liver'-cells are about 0.03 to 0.06 mm. deep in individuals about 1.0 mm. long.

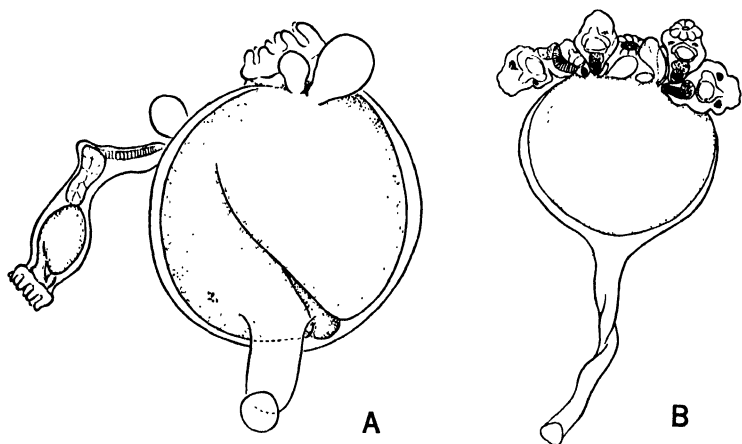
(2) In individuals with much enlarged stomach the calyx may be of two forms:

(a) Oval, with the stalk about one-half to two-thirds of the total length (Text-fig. 12). Of ten such individuals, measured with lophophore expanded, the average total length was 1.42 mm., with length of calyx 0.70 mm., and of stalk 0.72 mm.; the average width of the calyx was 0.42 mm., and that of the stalk 0.09 mm.

(b) Globular with stalk one-half or less of the total length (Text-fig. 13, A). Measurements of sixteen living individuals, with lophophore more or less closed, gave the following average measurements: total length

0.87 mm., with length of calyx 0.50 mm., and of stalk 0.87 mm.; width of calyx 0.44 mm.

A variation, apparently, of this type, is the form with the calyx much flattened dorso-ventrally, or disc-shaped (Text-fig. 13, B); this is a rare form and has only been seen in individuals of one small 'colony' found on a preserved Aphrodite.



TEXT-FIG. 13.

L. obesum. A. Living individual with large round stomach, and short stalk. Gonad immature, and not shown. $\times 57.25$. B. Individual with large stomach, but the calyx much flattened dorso-ventrally. Five buds are present on either side of the calyx. Preserved material. $\times ca. 41.4$.

In 'colonies' individuals with enlarged stomachs are mostly either of one form or the other; it possibly depends on the condition of the stomach.

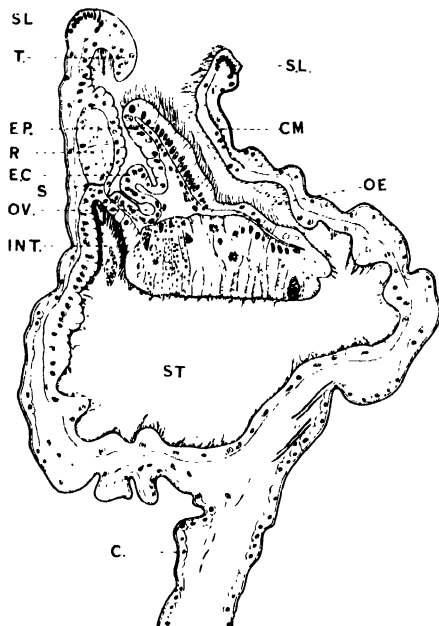
It is characteristic of *L. obesum* that in the greater number of 'colonies' examined, while most young individuals have the stomach more or less normal, with the so-called liver-cells not unusually developed, the medium and large specimens show an extraordinary development of these cells. They attain a great depth—0.09 mm. or more—and become crowded with granules, while the lobes extend proximally, the apical portion

of the stomach being very small (cf. Text-figs. 12 and 13 with Text-fig. 10). Owing to their extreme development the stomach is much swollen, occupying the greater part of the calyx, and the living animal appears as an opaque white or cream-coloured ball, easily visible to the unaided eye. When the animals are crushed, masses of clear granules of varying size stream out. The granules are on the whole discrete, but a number of oval or pear-shaped collections of granules or globules, 60μ to 80μ long, would appear to be contained in small cells.

The significance of this heaping up of, presumably, reserve products is not understood; it is suggested that it may be connected with the production of sex cells, and, or, with the reproduction of the animal by budding. It is found indiscriminately in males and females. While perhaps the majority of 'colonies' in this condition have been breeding or budding, some have had the gonad immature and with no indication of buds; on the other hand, at least one 'colony' with the stomach in a normal condition had some individuals carrying embryos in the vestibule, though the number carried simultaneously in this instance was very small—one only, with few exceptions, and that well developed; the gonad in the majority of these females contained only small or medium-sized ova. (Some individuals with immature gonad from this 'colony' are shown in Text-fig. 10.) That the great development of the 'liver'-cells is a normal phase in the life-history of *L. obesum* is evident from the fact that the majority of individuals, which may reach some hundreds or even thousands on each 'host', from 14 out of 20 infected *Aphrodite* were in this condition. *L. obesum* with highly developed 'liver'-cells have been found in February (8 out of 9 'colonies' in 1928), March (4 out of 4 'colonies' in 1930), and in September (2 out of 7 'colonies' in 1923).

Alimentary System.—The general form of the alimentary canal may be seen from Text-fig. 14. The oesophagus, which is lined with columnar, highly ciliated cells, showing well-marked ciliary rootlets, opens into the stomach about half-way down on its ventral face (Text-figs. 10 and 11), though the position varies somewhat. In most known species the opening of the oesophagus is nearer the proximal apex of the stomach,

as in *L. crassicauda* (Text-fig. 1, B, p. 325), *L. singulare* (Text-fig. 4, p. 337), and *L. claviforme* (Text-fig. 6, A, p. 345). Along the ventral face of the stomach there is a groove of low ciliated epithelium leading from the oesophagus to the apical region of the stomach, which in turn is continuous with



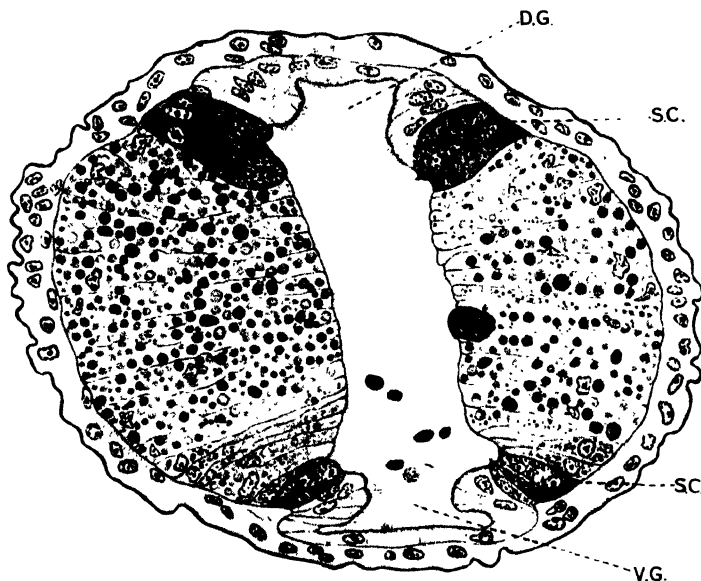
TEXT-FIG. 14.

L. obesum, female. Longitudinal section passing through the oesophagus, stomach, intestine, rectum (surface), and through the ventral and dorsal grooves, connecting the lumen of the oesophagus and intestine, respectively, with the apical region of the stomach. *C.*, cuticle; *CM.*, circular muscles of oesophagus; *EC.*, projection on floor of vestibule to which embryos become attached; *EP.*, epistome; *INT.*, intestine; *OE.*, oesophagus; *OV.*, oviduct; *R.*, rectum; *S.*, sphincter between intestine and rectum; *SL.*, sphincter muscles of lophophore; *ST.*, stomach; *T.*, tentacle. \times ca. 202.6.

the lumen of the intestine by a dorsal groove lined with a similar type of epithelium (Text-fig. 15). In forms in which the oesophagus opens low down on the ventral face of the stomach only

the dorsal groove is found, as in *L. saltans* (1, p. 128) and in *L. crassicauda*.

Narrow tracts of secretory cells are present on either side of the ventral and dorsal grooves, intervening between them and the lateral diverticula (Text-fig. 15); and are continuous round the apical region of the stomach, separating the cells of that region from those of the lateral lobes. In some species, notably



TEXT-FIG. 15.

L. obesum. Section transverse to the long axis of the body, passing through the stomach below the level of the entry of the oesophagus, and showing the ventral (*V.G.*), and dorsal (*D.G.*) grooves. On either side of the grooves are narrow tracts of secretory cells (*S.C.*). The 'liver'-cells contain many granules: a single gland-cell is present among the ends of the long 'liver'-cells of one side. The stomach was full of a coagulum which is not shown. Bouin's fixative; iron haematoxylin and acid fuchsin. \times ca. 430.

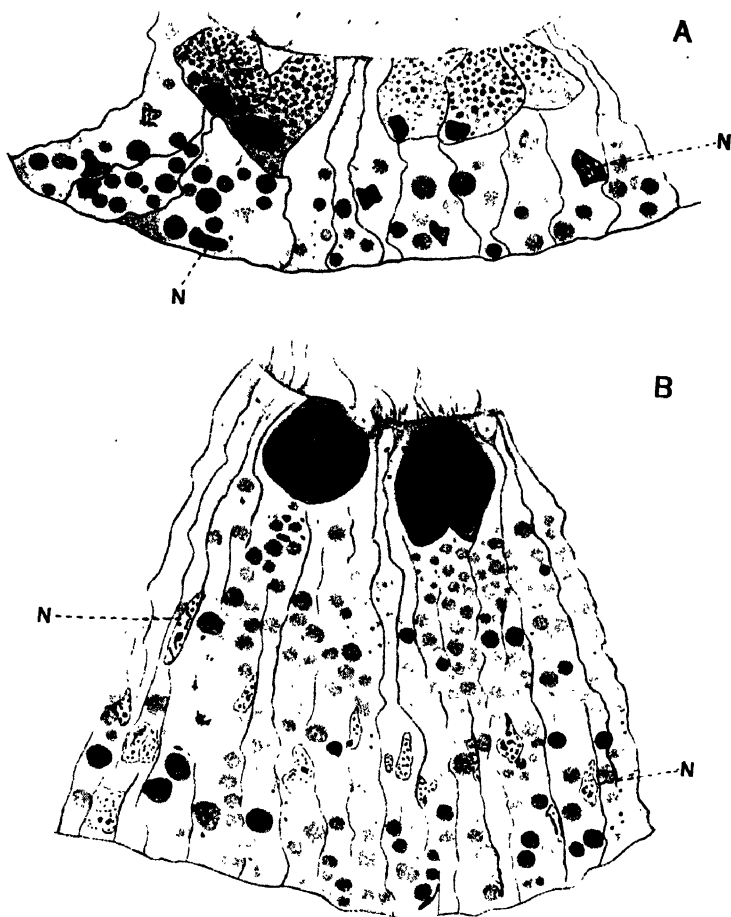
L. loxalina (1, p. 121), *L. saltans* (1, p. 129), and *L. davenporti* (23, p. 362) the two semicircular tracts surrounding the apical region appear to be highly developed, forming definite lobes. The appearance of the contents of the cells varies

in different phases of activity. In some cells they may be either finely or coarsely granular, staining with orange G, but not with Heidenhain's iron haematoxylin; in others the granules are larger, and stain darkly with haematoxylin; while in some the staining is so intense that separate granules cannot be distinguished. The contents of these cells appear not to be mucoid in character, as they do not stain with Mayer's mucicarmine or muchamaetin. They possibly secrete digestive enzymes. The nuclei are basal, large, round, and with most of the chromatin collected in centrally placed nucleoli.

In *L. crassicauda* there is a similar disposition of secretory cells, except that the ventral groove is short or absent.

The cells of the lateral lobes of the stomach are lowest where they pass into the tracts of secretory cells (Text-fig. 16, A), but in some parts may reach a great depth, 0.09 mm. or more, although narrow (Text-figs. 16, B; 17). Salensky (29, p. 9) gives the depth of the 'liver'-cells of *L. tethyae* as 0.001 mm.; in this species individuals are about 0.5 mm. long. In *L. crassicauda*, of which the length of average individuals is about 1.4 mm., they are about 0.025 mm. to 0.05 mm. in length.

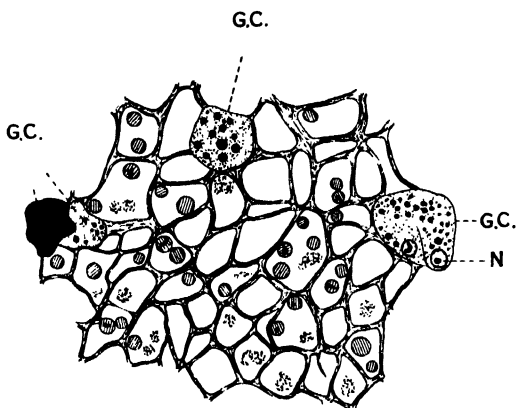
In specimens of *L. obesum* with the stomach much enlarged the so-called liver-cells are so crowded with coarse spherical inclusions of varying size that the cell-walls and nuclei are difficult to distinguish. The amount of cytoplasm present is extremely small, even when granules are few. The granules stain with varying intensity, most more or less uniformly, but less frequently some occur with a darker staining periphery, while others contain an irregular centre, staining darkly with haematoxylin (see Text-fig. 16). There is some tendency for the granules to collect towards the basal part of the cell. In some individuals very few vacuoles are present in the 'liver'-cells (resting phase?) (Text-fig. 16); in others they are numerous. The contents of these are of at least three kinds. Large vacuoles occur containing rounded masses of the same type of granule as is present free in the cell (V^1 , Text-fig. 18). Such masses are seen protruding beyond the free margin of the cells (Text-fig. 19, A), and there is no doubt that they are discharged,



TEXT-FIG. 16.

L. obesum. Longitudinal sections through the epithelium of the lateral diverticula of the stomach. $\times 735$. A. Short cells towards the edge of a diverticulum. Very few granules are present in the cells. Two groups of gland-cells are present, with granules staining with eosin. Bouin's fixative; Delafield's haematoxylin and eosin. B. Long cells, with very few vacuoles (resting-phase?). Two small vacuoles, with minute inclusions, occur near the free edge of the cells, and are probably secretory. The granules stain faintly with orange G, but some have centres staining intensely with iron haematoxylin. Between the ends of the 'liver'-cells are two small groups of gland-cells with granules staining intensely with iron haematoxylin. Bouin's fixative; iron haematoxylin and orange G. N, nucleus.

as similar collections of granules occur free in the lumen of the stomach. In this species, as in *L. davenporti* (23, p. 363), excretion is not confined to the rectal cells (see Assheton, 1, pp. 134-5). Other fairly large vacuoles, which seem to increase in size towards the base of the cells, contain a finely granular material, staining black with iron haematoxylin, which in sections appears as a fine semicircle (V^3 , Text-fig. 19, A). Possibly the greater part of the contents has disappeared during the



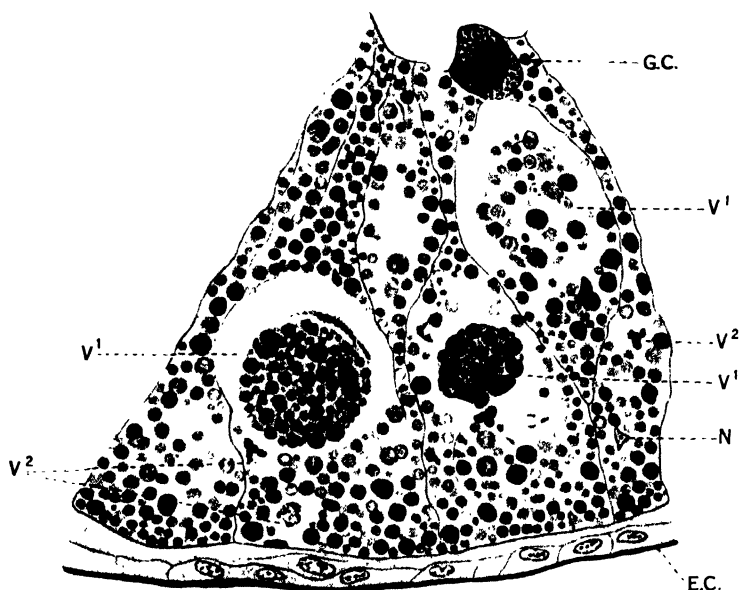
TEXT-FIG. 17.

L. obesum. Transverse section of 'liver'-cells, towards their free ends, passing through three groups of gland-cells (*G.C.*). Within the cells are granules, some with darkly staining centres, and small collections of fine granules staining grey with haematoxylin. *G.C.*, gland-cell; *N*, nucleus of gland-cell. Osmic fixation; iron haematoxylin and eosin. $\times 735$.

process of fixation and dehydration. Individuals which have many of these vacuoles would seem to have few of the first type. Finally, small vacuoles occur containing a small finely granular mass, staining intensely with Heidenhain's iron haematoxylin (V^2 , Text-figs. 18, 19, A). These may be seen at the free margin of the cells, and possibly contain secretory granules.

The nuclei are irregular in shape, with a small amount of scattered chromatin. They occur mostly in the basal third of the cells. The cells of the lateral diverticula of *L. loxalina* and *L. saltans* (see Assheton, 1, pp. 121 and 128) are said

to be unciliated, as are also those of *L. davenporti* (see Nickerson, 23, p. 362), while those of *L. crassicauda* (9 p. 276) are said to be ciliated. In *L. obesum* the 'liver'-cells appear to occur both in a ciliated and a non-ciliated phase: when the cells are actively excreting, cilia are absent, no doubt



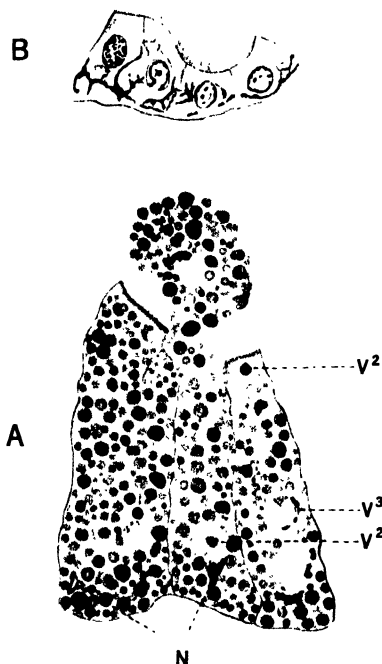
TEXT-FIG. 18.

L. obesum. Longitudinal section through the epithelium of a lateral lobe of the stomach. Two kinds of vacuoles are present: *V*¹, large vacuole containing large collection of very coarse granules; *V*², vacuole with finely granular contents staining intensely with iron haematoxylin; *G.C.*, gland-cell; *E.C.*, external cuticle of body; *N*, nucleus. Bouin's fixative; iron haematoxylin and acid fuchsin. $\times 735$.

having been shed or absorbed. It is possible that this may occur in the other species mentioned; it does occur in *L. crassicauda*.

The 'liver'-cells in the only four individuals of *L. crassicauda* sectioned had a very different appearance from those of *L. obesum*. Granules were exceedingly few or absent,

but this may be an unusual condition as the animals were living in a tank in the Laboratory, and therefore without abundant food. That granules do occur in the cells is evident from



TEXT-FIG. 19.

L. obesum. $\times 735$. A. Longitudinal section through epithelium of lateral lobe of stomach, to show extrusion of rounded mass of granules. N, nucleus; V², vacuole containing finely granular mass staining intensely with iron haematoxylin; V³, vacuole with crescentic arrangement of fine, intensely staining, granules. Bouin's fixative; iron haematoxylin and acid fuchsin. B. Longitudinal section through epithelium of apical region of stomach showing anastomosing strands, which stain intensely with iron haematoxylin. Bouin's fixative; iron haematoxylin and orange G.

Harmer's statement (9, p. 276). The cytoplasm was much more abundant than in the corresponding cells of *L. obesum*. The free ends of many of the cells were rounded, projecting

into the lumen, and it seemed as though they were being set free into the stomach. This is probably a secretory phase. In *L. obesum* a very few individuals have been observed with somewhat rounded ends to the cells; the cells contained exceedingly few granules, though the cytoplasm was still small in amount. In one *L. crassicauda* sectioned certain of the 'liver'-cells were dividing transversely, and the free half being shed. Of such parts of cells occurring free in the stomach, some at least contained a nucleus, or degenerating nucleus. An appearance as though parts of cells were being shed has been observed in *L. obesum*, though here the granules were so numerous that no nucleus was discernible, and it was difficult to distinguish with certainty such a condition from the excretion of large masses of granules.

In *L. obesum* short gland-cells occur in occasional groups of two or three, between the free ends of the long 'liver'-cells (Text-figs. 15-18). In these the rounded or oval nucleus is basal, and has a large nucleolus. These cells are not mucous-glands, as they are unstained by Mayer's mucicarmine or muchaematein. I have been unable to distinguish any mucous-glands in the alimentary canal of *L. obesum*, though Assheton (1, p. 128) found a few among the cells of the apical region of *L. saltans*, and mucus is said to be secreted in the stomach of *Pedicellina* (Cori, 6, p. 36).

The cells of the conical lower apex of the stomach are low and highly ciliated (Text-fig. 19, b); a similar type of cell lines its ventral and dorsal grooves. In life these cells contain small shining colourless globules; they blacken with Flemming's fluid and are probably fat globules. Assheton suggested for *L. saltans* that these cells were absorptive (1, p. 129). In material fixed in Bouin's picro-formol and stained with Heidenhain's iron haematoxylin, the cells, especially of the apical region and dorsal groove, of some individuals are seen to have anastomosing strands, or fibrils, staining intensely (Text-fig. 19, b), and in some individuals they are so numerous that the cells appear almost full of them.

The intestinal epithelium is densely ciliated, the cilia being thick and fairly short, and staining with iron haematoxylin.

The cytoplasm of these cells is denser than in other parts of the alimentary canal.

The side-walls of the rectum frequently contain large, pale yellow to orange, refringent, excretory spherules and fine granules. So far as could be ascertained the cilia are not restricted to the ventral and dorsal walls as in *L. saltans* (1, p. 133). In embryo-carrying females the rectum is of great length, stretching across the roof of the enlarged vestibule to open in its normal position (Text-fig. 12, II, A, p. 358).

Excretory System.—A variable number of large excretory or accretory cells (see p. 327), up to twelve or more, are present in the calyx on either side of the oesophagus (Text-fig. 11, c, p. 357). On a trace of neutral red being added to the seawater, the granules in these cells after a time become dark red: with methylene blue they take a blue tint.

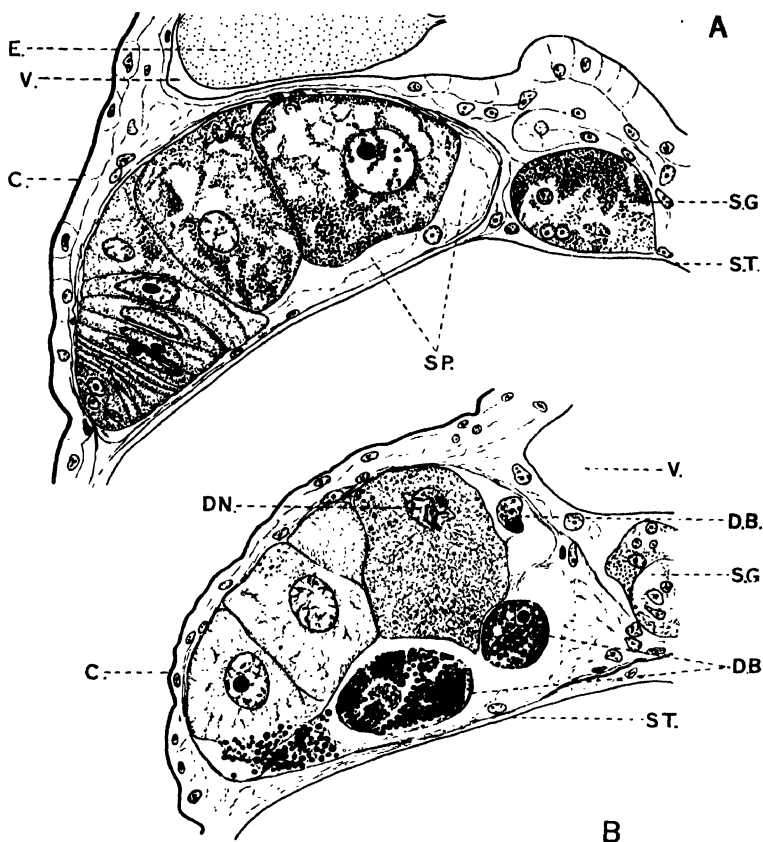
True nephridia were not observed in the living animal, probably owing to the difficulty of observation, but indications of them were seen in sections.

L. obesum has no special organs of sense: a nerve ganglion is present in the usual position, but nerves cannot be traced in the living animal as can be done in *L. crassicauda*.

Reproductive System.—The sexes, so far as is known at present, are separate, but few sections (about fifty animals) have been examined, and in the living animal degenerating sperm or ova would be difficult to identify. The gonad is paired: the mature testes may reach a large size; the mature ovary is relatively smaller. Spermatozoa when crushed out of the gonad, or vesicula seminalis, are either motionless or undulate slowly. They are thin, with fine tails, and are about 45μ long.

In living individuals in which the calyx has become blown out, as a result of the degeneration of the gelatinous matrix, the muscles can be seen clearly. Owing to the degeneration of the most mature ova rendering it visible, the ovary is seen to be surrounded by a delicate structureless membrane, to which muscle-fibres are attached.

In young females before reproduction is in full swing there appears to be only one well-developed ovum, and several small or tiny developing ova in the gonad. The peculiar flattened



TEXT-FIG. 20.

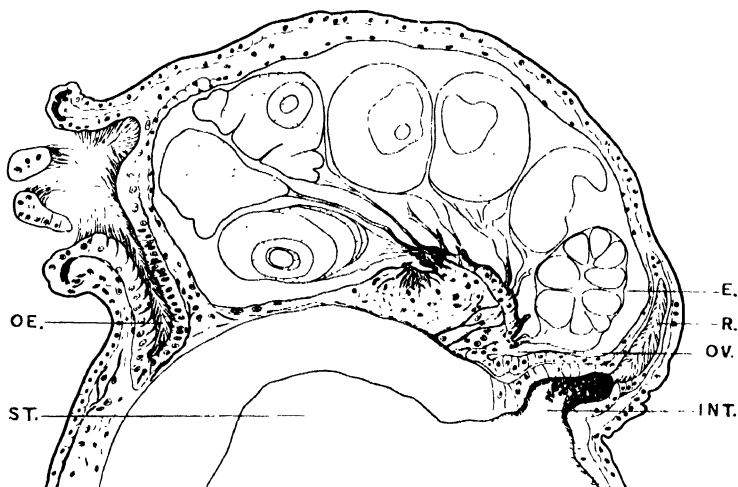
L. obesum. A. Obliquely longitudinal section through ovary, in which two large ova are present; the space (SP.) indicates that a ripe ovum has not long been extruded. The flattened and tiered arrangement of the young ova at the lower, blind end of the gonad is noteworthy. Flemming's fluid without acetic acid; iron haematoxylin and acid fuchsin. B. Section through ovary to show presence of degenerating bodies (D.B.) composed of globules staining black with iron haematoxylin, as do the yolk globules. Three ova of considerable size are seen in the section; the nucleus (D.N.) of the largest is degenerating. Bouin's fixative; iron haematoxylin and acid fuchsin. C., external cuticle of calyx; E., embryo; S.G., shell-gland; ST., outline of stomach; V., vestibule. $\times 342.5$.

and tiered arrangement of the young ova at the lower, blind end of the gonad is shown in Text-fig. 20, A. In individuals reproducing rapidly there may be four to six good-sized ova in each side of the gonad, though the oldest is distinguished by its greater size and greater opacity. When it is considered that the vestibule may contain as many as twenty-four to twenty-six embryos at one time, it is understandable that ova are likely to be approaching ripeness in fairly rapid succession. The ripe ovum is about 0.1 mm. in diameter, heavily yolked and opaque. As noted by Vogt for *L. phascolosomatum* (36, p. 326) an ovum attains ripeness on either side alternately. Rounded bodies have been observed in the ovary of living specimens, which did not appear to be ova; they had a definite dark outline—like that of a bubble—and contained a number of shining globules of varying size. As many as six have been seen in one-half of the gonad, varying from 0.02 mm. to 0.05 mm. in diameter. These were observed in individuals which had been kept in finger-bowls for several days, and it is possible that they were derived from degenerating ova. Similar bodies have been seen in the ovary of living *L. claviforme*. It may be noted, however, that Harmer (9, p. 282) described in *L. tethyae* the developing ovum devouring curious masses, which he considered play the part of a vitellarium. In two or three of the individuals of *L. obesum* sectioned, several rounded masses of globules, or granules, staining black—as do the yolk globules—with iron haematoxylin, were present in the ovary (*D.B.*, Text-fig. 20, B). It is probable that these had resulted from degeneration of ova, especially as large ova with degenerating nuclei have been observed (*D.N.*, Text-fig. 20, B).

In connexion with the oviducts is a pair of large pear-shaped glands, which doubtless secrete the delicate vitelline membrane, found surrounding the embryo in the vestibule. Ducts leading from these glands open into the median oviduct near the entry of its two lateral ducts from the gonads.

The embryos, like those of *L. phascolosomatum*, remain in the vitelline membrane until they are fully formed larvae, and leave the parent on the rupture of the membrane. The embryos are attached to a small projection (present only

in the female) on the floor of the vestibule, between the epistome and the oviduct, by the drawn-out continuations of their envelopes (Text-figs. 21, 22). A similar projection would seem to be present in *L. loxalina*, from a consideration of Assheton's fig. 5, Pl. 7 (1): a somewhat similar condition is seen in *Pedicellina cernua* (6, p. 26). The envelopes are

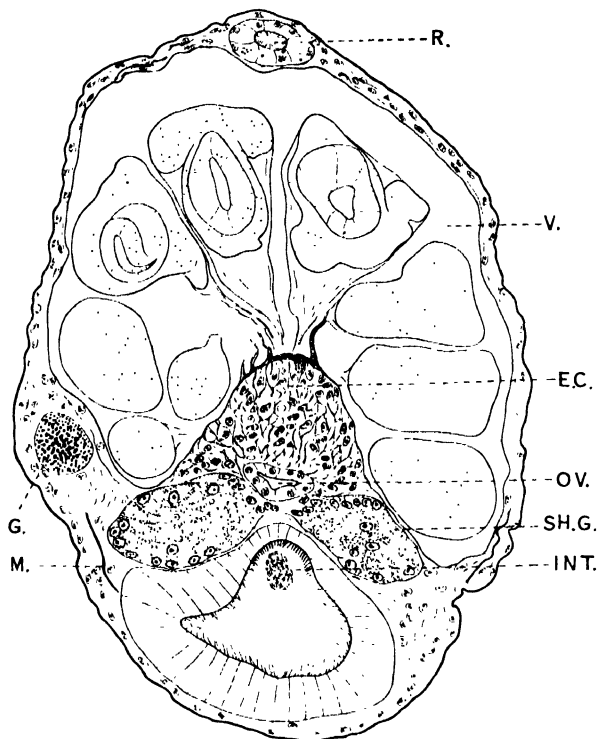


TEXT-FIG. 21.

L. obesum. Longitudinal section through the vestibule of a female, showing the attachment of the embryos by the drawn-out continuation of their envelopes, to a projection on the floor of the vestibule. The youngest embryo (*E.*) is nearest the oviduct (*OV.*). *INT.*, intestine; *OE.*, oesophagus; *R.*, rectum; *ST.*, stomach. Flemming's fluid without acetic acid; iron haematoxylin and acid fuchsin. $\times 171.4$.

sufficiently large for the fully formed larvae to move around in them. Normally a single embryo is contained in each envelope, but on two occasions in different individuals, two embryos, with ciliated ring developed, were enclosed in one vitelline membrane. This perhaps is liable to occur during rapid extrusion of ova into the vestibule. Immature larvae are occasionally forced out of the vestibule by violent contraction of the parent on being disturbed; in such instances they are generally attached together by their stalks in groups of three.

Adult females have the vestibule much enlarged and crowded with embryos (Text-fig. 12, II, A, p. 358), in various stages of development. The number carried simultaneously, which may reach at least twenty-six, appears to be unusually large for the

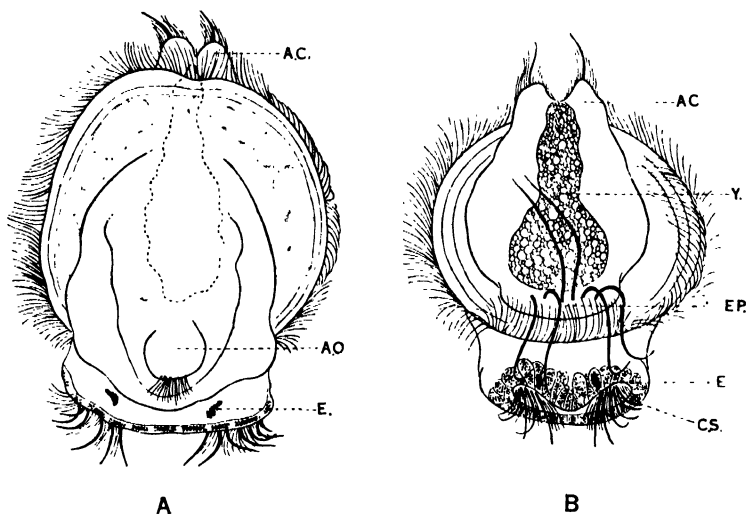


TEXT-FIG. 22.

L. obesum. Oblique section of the vestibule, passing through the embryo-carrier, the oviduct, and the shell-gland. *E.C.*, embryo carrier; *G.*, ovary of one side; *INT.*, intestine; *M.*, muscle-fibres; *OV.*, oviduct; *R.*, rectum; *SH.G.*, shell-gland; *V.*, vestibule. Bouin's fixative; iron haematoxylin and acid fuchsin. $\times 200$.

family, though there is little information as to the maximum number carried in the different species. As many as nine or ten have been seen in the vestibule of a specimen of *L. singulare* 0.5 mm. long (measured mounted, with lophophore

closed) from the elytra of *Aphrodite* at Plymouth. This is a large number considering the small size of the species and the size of the ova (about 0.08 mm. to 0.09 mm. in diameter). *L. cirriferum* (12, p. 14), with an average total length of 0.650 mm., carries as many as six or seven embryos simultaneously



TEXT-FIG. 23.

L. obesum. Sketches of living, free-swimming larvae. A. Aboral view; B. oral view. A.C., anal cone; A.O., apical organ; C.S., ciliated sacs of dorsal organ; E., eye-spot; EP., epistome bearing long, stout cilia and covering the mouth; Y., yolk-mass in alimentary canal. $\times 274$.

in the vestibule. In *Loxocalyx leptoclini* (9, p. 288), which has an average total length of about 0.5 mm., the number of embryos in the vestibule 'seldom exceeds about 2 or 3'. The small number is, however, probably correlated with the specialized method of nutrition of the embryos in this species.

Larva.—The larva¹ of *L. obesum* (Text-fig. 23) resembles

¹ Sir S. F. Harmer has pointed out to me that it also has a striking resemblance in the attitude shown in Text-fig. 23, B, to a figure (Pl. xvi, fig. 14) of the organism which was described by Busch (1851, 'Beobachtungen über Anatomie und Entwicklung einiger wirbellosen Seethiere',

that of *L. singulare*. The eye-spots are a reddish brown; they are sometimes unequally divided into two. The stiff cilia or sense-hairs of the apical organ ('sucker', 'ciliated disc') are motionless.

The six long stout cilia on the epistome have a slow rate of beat, though the rate varies somewhat; the effective beat is backwards, that is, towards the anal cone. At times they are held motionless, directed backwards. These cilia are compound in structure. The epistome is mobile; it may project over the mouth as in Barrois' figure of *L. singulare* (3, Pl. 1, fig. 21), or may be bent posteriorly.

The floor of the vestibule, including the epistome and anal cone, is ciliated.

The six, or so, stout cilia present among the fine cilia of the ciliated sacs of the dorsal organ also have a slow rate of beat. They do not all beat in the same direction; the inner one of each sac or depression beats roughly across the length of the organ; the two to the outer side of these beat along the length of the organ—the effective stroke being outwards; while the two or three shorter ones at the outer ends of the sacs appear more or less motionless.

The long cilia of the corona beat inwards towards the vestibular cavity; when the corona is in a state of partial contraction the cilia lie motionless directed upwards, as in Barrois' Pl. 1, fig. 13, of *L. singulare* (3). When entirely retracted the corona is reflected to the interior of the vestibular cavity, and the cilia are almost entirely hidden. A larva was observed on one occasion to stop suddenly, while swimming, with the corona fully extended, and the cilia motionless, radiating outwards. While the larva is still within the vitelline membrane, the beat of these cilia is slow, and more or less synchronous over much of the corona, no doubt owing to the constraint of the surrounding membrane. Synchrony of beat is lost in the free-swimming larva; the beat becomes much more rapid, but the rate of beat appears to be under the control of the animal.

p. 132, Pl. xvi, figs. 12–16. Separately published, Berlin) as *Cyclopelma longociliatum* and later identified by Barrois (3, p. 5) as a *Loxosoma* larva.

The method of progression of the larva varies; at times it swims forward evenly with the dorsal organ, with the eye-spots, foremost, or it may progress by a series of somersaults, while at other times it whirls round and round, keeping more or less in the same spot. Alteration in the method of progression is possibly due to differences in the relative strength of beat of the coronal, and the epistomal cilia. It is possible, also, that changes in the position of the epistome, by causing slight alteration in the general direction of beat of the epistomal cilia, may result in the alteration of the direction of movement of the larva.

Larvae were observed to anchor themselves temporarily, by the oral surface, the coronal cilia beating slowly, while the region with the dorsal organ, protruded to its fullest extent, explored in all directions; when it faced directly upwards, the eye-spots were clearly visible on the floor of the ciliated sacs; in any other position they are visible through the transparent walls of the sacs. While in this position the larva may move slowly forwards by the contraction and expansion of the ciliated corona, which is now elongated and oval in outline, and perhaps helped by clawing movements of the large cilia of the epistome.

Large mucus-glands are present on the floor of the vestibule (as shown by staining with Mayer's mucicarmine and mucachæmatin), though mucus-glands could not be demonstrated in the adult.

Larvae on one occasion lived at least six or seven days after hatching without alteration in form. It would appear that in this *L. obesum* resembles *L. tethyae*, which Harmer (9, p. 304) kept alive for eight days after hatching, 'at the end of which time they were still free-swimming, and externally at any rate, showed no obvious indications of buds'. The larvae of *L. leptoclini*, on the other hand, produced a pair of buds within four days (see Harmer, 9, p. 300).

Females carrying embryos in the vestibule have been seen in February, March, April, and September: no specimens have been examined during the summer months.

Buds.—The buds are situated on either side of the calyx, just below the lophophore (Text-fig. 13, p. 360). They are found on individuals of both sexes, and on females with the

vestibule crowded with embryos. The number of buds may be large, as many as six on each side, though a smaller number is perhaps more common, many adults having two buds on one side, and one on the other: the number present doubtless depends on general conditions. So far the large number has only been found among large individuals of two small 'colonies' (one taken in February, and the date of the other unknown) in which the gonad was immature or not distinguishable. When a large number of buds is present, there is only a slight difference in size between buds of successive ages (Text-fig. 13, B, p. 360).

Nickerson (23, p. 369) says of *L. davenporti* that there may be as many as six buds a side, but more commonly two, three, or four upon a side. Five or six buds a side have been described for *L. kefersteini* (5, p. 30): *L. crassicauda* has three or four a side, but the majority of known species appear generally to have a small number of buds.

Buds may reach a length of about 0.55 mm. before they become free. As previously mentioned they possess a large foot-gland and groove.

Individuals reproducing by budding have been observed among infections examined in February, March, April, and September. No specimens were examined during the summer months.

Proportion and Size of the Sexes.

Females have been found very greatly to preponderate over males in infections in which the sex of individuals is easily recognizable, that is, in a population composed mainly of sexually mature, or nearly mature, individuals; but more information is needed, especially of infections in which the gonad of the majority of individuals is immature, though the individuals are large, for among some of these—judging, however, by only one sample—the males may exceed the females in number (see Table II, 2). When the number of males is small, a very high percentage of them is fully mature. Only few infections have been examined in any detail; in three out of the four, females predominated.

TABLE II.
Sex Proportions in Random Collections of *Loxosoma obesum* from four Aphrodite aculeata.

Date.	♂		♀		♂		♀		♂ mature. ¹		♀ mature. ¹		Total.	Remarks.
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
1. February 7, 1928	0	(0)	90	(100)	0	(0)	0	(0)	0	(0)	8	(8.8)	90	Majority with gonad of median size, but not fully mature. Smallest female with embryos, 1.35 mm. long. Females 0.6-2.1 mm. long.
2. February 20, 1928	77	(48.43)	48	(30.19)	34	(21.38)	37	(48.05)	6	(12.50)	159		159	Majority of females with small, immature gonad. Smallest female with embryos, 0.96 mm. long. Smallest mature male 0.86 mm. long. Males 0.46-1.9 mm.; females 0.6-1.6 mm.; sex indeterminate 0.4-1.5 mm. long.
3. March 12, 1930	4	(3.45)	110	(94.83)	2	(1.72)	4	(100)	52	(47.27)	116		116	No measurements made.
4. March 19, 1930 i-iii ²	21	(17.21)	99	(81.15)	2	(1.64)	21	(100)	48	(48.48)	122		122	Smallest female with embryos, 0.87 mm. long. Smallest mature male 0.68 mm. Males 0.68-2.2 mm.; females 0.56-2.2 mm.; sex indeterminate 0.6-0.7 mm. long.
iv	17	(6.88)	230	(93.12)	0	(0)	17	(100)	?	(?)	247		247	Females not measured, and no note made of the number with embryos. Smallest mature male 0.54 mm. long. Males 0.54-1.84 mm. long.

¹ 'Mature', in the male, is determined by the presence of sperm in the gonad or vesicula seminalis; in the female by the presence of an embryo in the vestibule.

² i-iv are small groups of *Loxosoma* taken from different parts of the dorsal surface of the 'host' and examined separately.

Nickerson (23, p. 364) and Kowalewsky (18, p. 5), for *L. davenporti* and *L. neapolitanum* respectively, noted the relative scarcity of males, and Claparède (5, p. 30) states that in *L. kefersteini* from the Gulf of Naples the only individuals showing sexual organs were females, while Vogt (36, p. 326) noticed that the tufts or 'colonies' of *L. phascolosomatum* on *Phascolosoma* were to a great extent either male or female. A similar condition has been observed for *L. phascolosomatum* on the shells of *Lepton* and *Mysella* in the Salcombe Estuary (2, p. 751). The curious state of affairs existing among *L. crassicauda* in a tank in the Plymouth Laboratory, in which all individuals in which sex was recognizable were in the male condition during the eleven months of observation, is discussed on p. 334.

Some figures of infections are given in Table II; it is probable that males might have been found among the infection of February 7, 1928, if more specimens had been examined. In addition to the data given in Table II, it was observed that of the individuals infecting an *Aphrodite* on April 20, 1928, there were fewer males than females, and that most of the females had the vestibule crowded with embryos.

When it is taken into account that in heavy infections there may be thousands of individuals on one *Aphrodite*, the number examined and tabulated (see Table II) is comparatively very small.

It was hoped by measurements of the two sexes to reach some conclusion as to whether the sexes are separate, or whether a change of sex occurs. A belief in the latter occurrence has been expressed by Harmer (9, p. 280) and by Nickerson (23); the latter writer actually found a specimen with male and female elements in the same gonad, the male state replacing the female.

Nickerson (23, p. 367) says that the males he studied (about ten) were of nearly average size, while all exceptionally large individuals, specimens 2.0 mm. or more in length, were without exception females. He suggested—considering this fact in conjunction with specimens in which male elements were replacing female in the gonad—that several periods of sexual activity,

alternately male and female, occurred in the life of a single individual.

Prouho (27, p. 107) found in *L. annelidicola* that the males were larger than the females, though he did not consider he had examined sufficient numbers to establish the law in his species.

Harmer (12, p. 15) writing of *L. cirriferum* says 'Male specimens are usually smaller than females, although I have found a male almost as large as the largest females. I find no evidence of protandry; and eggs may be produced by females which are no larger than the male shown in Text-fig. 13.'

Probably the number of *L. obesum* examined is too small to prove anything one way or the other. Such measurements as have been made indicate that there is no appreciable difference in size between the sexes; this would be consistent with either a separation of the sexes, or with the occurrence of several periods of sexual activity, alternately male and female, in the life of a single individual.

In a sample¹ from an Aphrodite obtained on March 19, 1930, the average total length of the females was slightly greater than that of the males; the figures were calculated on ninety-one females, thirty-six males, and two individuals in which the gonad was too small for the sex to be determined, and are as follows:

	<i>Female</i> (including 46 with embryos).	<i>Male.</i>	<i>Sex</i> <i>indeterminate.</i>
Average total length	1.237	1.165	0.65
Average length of calyx	0.604	0.481	0.38
Average length of stalk	0.633	0.684	0.27
Average width of calyx	0.378	0.342	
	(on 37 females)	(on 26 males)	

Text-fig. 12 (p. 358) is of individuals from this infection.

¹ This is the same sample, 4, as in Table II, except for 10 individuals which had broken stalks, and 230 females, which were not measured.

In a sample¹ from an *Aphrodite* obtained on February 20, 1928,² the average length of male and female were reversed, the length of the male being slightly greater than that of the female. Actual figures are as follows, calculated on sixty-nine males, forty-two females, and thirty-four individuals in which sex could not be determined (see Table II, p. 379, for the characters of this infection):

	<i>Female</i> (including 4 with embryos).	<i>Male.</i>	<i>Sex</i> <i>indeterminate.</i>
	mm.	mm.	mm.
Average total length	1.055	1.156	0.950
Average length of calyx	0.571	0.594	0.506
Average length of stalk	0.484	0.562	0.444
Average width of calyx	0.451	0.484	
	(on 48 females)		(on 68 males)

If these two samples are taken together—a total then of only 274 individuals, of which 133 are female, 105 are male, and the sex of 36 indeterminate—the average total length of male and female is very close, the male being a trace larger than the female.

	<i>Female.</i>	<i>Male.</i>
	mm.	mm.
Average total length	1.146	1.160
Average length of calyx	0.587	0.537
Average length of stalk	0.559	0.623

The sample of March 1930 shows a difference in the proportions of the calyx in the two sexes, but in that of February 1928 there is little difference. The difference in the 1930 sample

¹ This is the same sample, 2, as in Table II, with the exception of fourteen individuals with broken stalks.

² Measurements of March 19, 1930, were of individuals with the lophophore open, those of February 20, 1930, were of specimens with the lophophore closed, but as in the former instance the tentacles were not included, the measurements are roughly comparable. The *Loxosomas* of February 20, 1930, were not narcotized, but care was taken that they were uncontracted before measuring.

may probably be explained by the fact that this one included a large number of females with embryos in the vestibule (46 out of 91, 50.55 per cent.), and the enlargement of the vestibule of the embryo-carrying female tends to increase the length of the calyx in proportion to the width. In the February 1928 sample only four of the twenty-four females had embryos in the vestibule.

It might be noted that in the male the large mature gonad tends to displace the ventral part of the lophophore towards the free end, so that the lophophore becomes almost terminal in position (see Text-fig. 12, I. p. 358).

From a consideration of measurements and sex it may perhaps be concluded that the male reaches maturity at a smaller size than does the female. The smallest mature male among the sample from the infection of March 19, 1930, was only 0.54 mm. in total length, while the smallest mature female with one embryo in the vestibule was 0.87 mm. in total length; the presence of sperm in the male, and an embryo in the vestibule of the female being taken as evidence of maturity.

An analysis for sex and size of samples of *Loxosoma* from two *Aphrodite* of February 20, 1928, and March 19, 1930, are given in Tables III and IV; these are, except for the omission of individuals with broken stalks, the same samples as in Table II. The figures, inconclusive as they are, are given in some detail, as previous notes on the proportion and size of the sexes in *Loxosoma* seem to have been based on the examination of very few individuals.

Discussion of Relationships.

There would appear to be no doubt of the specific distinctness of *L. obesum*. It differs from most, if not all known species with eight, or about eight, tentacles, in the much larger size it attains, in the constancy of the number of its tentacles, and in the small size of its lophophore. Very few species have been described as having practically always eight tentacles; one of these is *L. nitschei* Vigelius (35). This form was originally described from *Menipea ternata*, Barents Sea, when its height was given as 0.15 mm.; the measurements were made, however, on badly preserved material, and Vigelius's figures and

TABLE III.

Analysis for Sex and Length of a Random Collection¹ of 145 *Loxosoma obesum* from Different Parts of the Dorsal Surface of one Aphrodite, February 20, 1928.

<i>Length in mm.</i>	0.25-0.5.	0.5-0.75	0.75-1.0	1.0-1.25.	1.25-1.5.	1.5-1.75.	1.75-2.0.	<i>Totals.</i>
Males, number:								
(a) Immature	(1)	(3)	(10)	(9)	(10)	(4)	(1)	
(b) Mature (sperm present)	—	—	(7)	(12)	(7)	(4)	(1)	
(a) and (b)	1	3	17	21	17	8	2	69
Females, number:								
(a) Without embryos	—	(4)	(6)	(25)	(3)	—	—	
(b) With embryos	—	—	(2)	(1)	—	(1)	—	
(a) and (b)	—	4	8	26	3	1	—	42
Sex indeterminate, number	2	5	8	15	4	—	—	34
Totals	3	12	33	62	24	9	2	145

¹ This is the same sample as in Table II, less 8 males (6 mature) and 6 females (2 with embryos) with stalks broken.

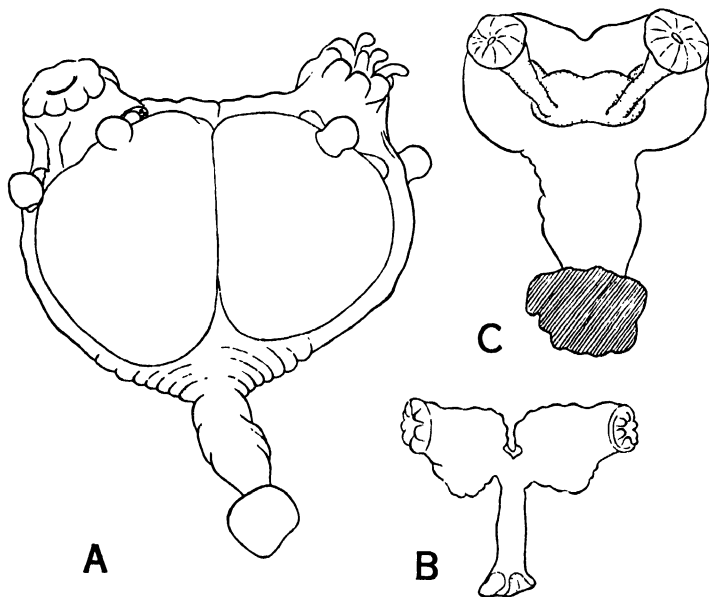
TABLE IV.

Analysis for Sex and Length of a Random Collection¹ of 116 *Loxosoma obesum* from Different Parts of the Dorsal Surface of one Aphrodite, March 19, 1930.

<i>Length in mm.</i>	0.5-0.75.	0.75-1.0.	1.0-1.25.	1.25-1.5.	1.5-1.75.	1.75-2.0.	2.0-2.25.	<i>Totals.</i>
Males, number (all with sperm present)	2	4	6	3	2	1	2	20
Females, number:								
(a) Without embryos	(4)	(23)	(17)	(4)	—	—	—	
(b) With embryos	—	(7)	(10)	(4)	(11)	(5)	(9)	94
(a) and (b)	4	30	27	8	11	5	9	
Sex indeterminate, number	2	—	—	—	—	—	—	2
Totals	8	34	33	11	13	6	11	116

¹ This is groups i-iii of sample 4 in Table II, less 1 male and 5 females with stalks broken.

description were necessarily inadequate. It was rediscovered by Roper (28, pp. 56, 57), growing in great abundance on algae, hydroids, and polyzoa, &c., in a tank in the Dove Marine Laboratory at Cullercoats, Northumberland, but in her short note little was added to its description. Harmer (12, p. 20)



TEXT-FIG. 24.

Double specimens of *Loxosoma*. A, B. *L. obesum* (preserved specimens). \times ca. 76.3. C. *L. singulare* (living specimen). The end of the stalk is hidden by a collection of debris. \times ca. 93.3.

mentions that *L. nitschei* from Cullercoats may reach a total length of 0.59 mm. when fully expanded. From what is known of *L. nitschei* it is extremely improbable that the Plymouth form can belong to that species. *L. obesum* is distinguished from *L. nitschei* by its much greater size and its general form (*L. nitschei* is said to be short and compact (35)). Other species which have eight tentacles are *L. murmanica* and *L. brumpti*, from the anterior and posterior ends, respectively, of *Phascolion spitzbergense* found

in the Kola Fjord, on the Murman coast of the Barents Sea; the former species, however, has the stalk and proximal part of the calyx covered with a thick brown cuticle, and the latter is provided with two prominent sense- (?) organs (24). Two species from Malay waters having eight tentacles are *L. sluiteri* and *L. subsessile* (12, pp. 5, 9, 19); the former is found on *Phascolion convestitus* and reaches a length of 0.4 mm., the latter occurs on *Conescharrellina* and is only 0.12 mm. in length. Size and form distinguish these two species from *L. obesum*.

L. obesum differs from all known species of *Loxosoma* (so far as the life-histories of these are known at present) in the peculiar enlargement of the stomach, which is found in perhaps the majority of individuals.

Double Specimens.

Four double specimens of *L. obesum* have been seen, which showed two different degrees of union:

1. Two specimens had a common stalk, and the bodies united side by side, with the ventral surfaces facing in the same direction. The two lophophores were distinct; each individual had its own reproductive organs and, in the specimen-carrying buds (Text-fig. 24, A), separate budding zones. The two digestive systems appeared to be separate, though the stomachs were in close contact, causing slight alteration of shape. These double *Loxosoma* were not sectioned, and in one specimen it was impossible to determine, from the entire preparation, whether the small reproductive organs were male or female, and whether they were all alike. In the second specimen all four reproductive organs were female. The condition of the nervous system could not be determined in one specimen; in the other the nerve ganglions of the two individuals were separate. The lophophores were turned outwards: in the specimen carrying buds the two outer budding zones were on the extreme edge of the calyx, that of the left individual being just on the dorsal surface. These specimens in their degree of

union are very similar to the first one described by Nickerson (22).

2. Two specimens showed a lesser degree of union. The stalk was single except for a short distance at the apex, but the bodies were entirely separate. One of these (Text-fig. 24, B) was much smaller than the other. These showed a slightly more advanced degree of union than the second specimen described by Nickerson.

Double *Loxosoma* have been previously described by Nickerson (22) as occurring among normal individuals of *L. davenporti*, and their origin discussed by him.

The work recorded in this paper and in the following one on 'The Ciliary Feeding Mechanism of the Entoproct Polyzoa, &c.', was carried out at the Marine Biological Association's Plymouth Laboratory, to the Director and Council of which I desire to express my gratitude; part of it was done during the tenure of a research studentship of Bedford College, University of London.

I am greatly indebted to Sir S. F. Harmer, F.R.S., for reading the manuscript, for making valuable suggestions, and for certain references to the literature. My thanks are also due to Dr. E. J. Allen, F.R.S., for reading the manuscript and for the interest he has taken in the work; and to Miss M. A. Sexton for help with the translation of German references.

SUMMARY.

Four known species of *Loxosoma*, namely, *L. phascolosomatum* Vogt, *L. crassicauda* Salensky, *L. singulare* Keferstein, and *L. claviforme* Hincks, and a new species *L. obesum* are found in the Plymouth region, and are described.

L. phascolosomatum is found on *Phascolosoma vulgare*, and in addition on two molluscs, *Lepton clarkiae* and *Mysella bidentata* from the burrows of *Phascolosoma (pellucidum) elongatum* from the Salcombe Estuary.

L. crassicauda lives in the tanks in the Laboratory. Its average length is 1.4 mm. Between March 1929 and February 1930 males only were found: no ova were seen.

L. singulare.—Occurs on *Aphrodite aculeata*; it varies between 0.18 and 0.8 mm. in length. In females carrying embryos the vestibule has two diverticula, one on either side of the rectum.

L. claviforme.—It is considered a valid species, and may be distinguished from *L. singulare* by: (1) its greater size and length of stalk, (2) greater number of tentacles (commonly twelve), (3) position of the budding zone, and (4) the presence of paired sense-organs. Its average length is about 0.8 mm. It occurs on *Hermione hystrix*.

A small group of *Loxosoma*, found on *Aphrodite aculeata*, were intermediate in form between *L. singulare* and *L. claviforme*, and were peculiar in retaining a number of their buds. The sex of such buds in several instances differed from that of the parent.

L. obesum sp. nov. is found on the dorsal surface of *Aphrodite aculeata*. It may reach a length of 2.4 mm.; average individuals are rather more than 1.0 mm. in length. The lophophore is small, and bears almost invariably eight tentacles. Longitudinal muscles only are present in the stalk, which ends in a small disc of attachment. A foot-gland is present in the bud, and is frequently preserved as a vestige in the adult. The buds are near the lophophore, and may be as many as six on either side. The larva resembles that of *L. singulare*.

Two main forms may be distinguished, differing in shape of the calyx and development of the stomach.

The ovary may contain six well-developed ova on either side, and the vestibule twenty-six embryos.

With one exception, females greatly exceeded males in number, and it is probable that the male becomes sexually mature at a smaller size than does the female.

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The Ciliary Feeding Mechanism of the Entoproct Polyzoa, and a comparison with that of the Ectoproct Polyzoa.

By

Daphne Atkins, B.Sc.

With 12 Text-figures.

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INTRODUCTION.

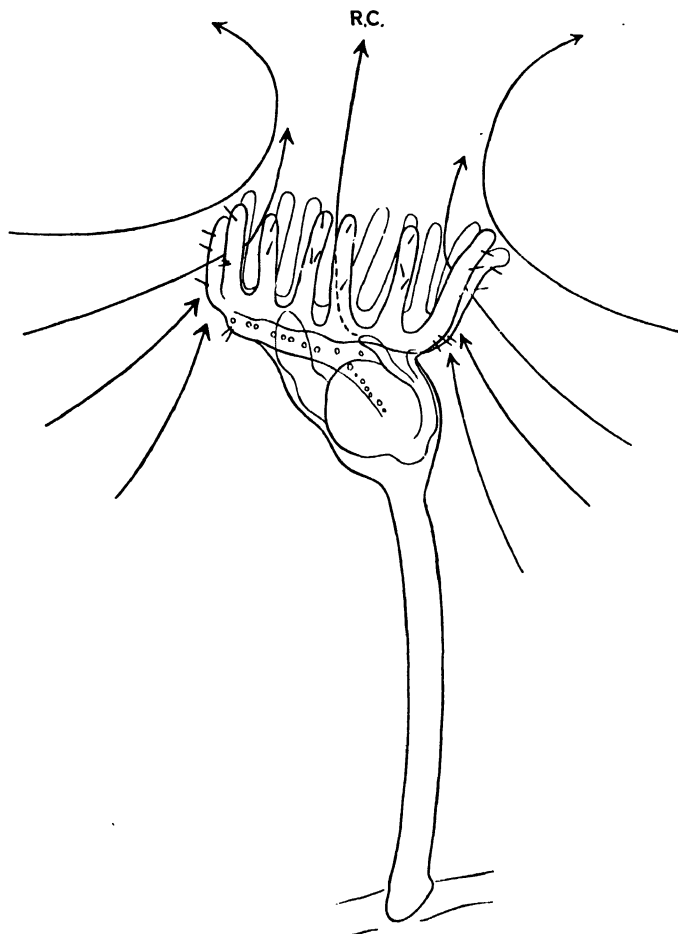
THE ciliary feeding mechanism of the Entoproct Polyzoa does not seem to have been worked out in any detail, as has that of the Ectoproct Polyzoa (4, 5, 18), although certain references to it occur in the literature of the group (27, 9). The following account of the ciliary feeding mechanism of the Entoprocta is based chiefly on an investigation of *Loxosoma*, though *Pedicellina* was also observed. *L. crassicauda* was chosen for the greater part of the work on account of the large size of its lophophore; the method of feeding of the other species of *Loxosoma* found at Plymouth, namely, *L. singulare*, *L. claviforme*, *L. phascolosomatum*, and *L. obesum* (3), is, however, identical.

THE STRUCTURE OF THE LOPHOPHORE AND OF THE
TENTACLES IN THE ENTOPROCTA.

In *Pedicellina*, as is well known, the plane of the lophophore is at right angles to the main axis of the stalk and calyx; in *Loxosoma* the lophophore is set obliquely. In at least some species of *Loxosoma*, however, the dorsal half of the lophophore is generally bent backwards during feeding, and the lophophore is then practically at right angles to the calyx (Text-fig. 1) (see also Assheton on *L. saltans* 2, p. 125, and Pl. 6, fig. 10). The numbers of tentacles springing from the lophophore varies in the different species of *Loxosoma*, and is generally somewhat variable within the species. The smallest number known to be present is eight, as in *L. nitschei* (25), *L. obesum* (3), &c., and the largest number twenty-nine in *L. davenporti* (19). In *Pedicellina cernua* the number is fourteen to twenty-four. In *Loxosoma* and *Pedicellina* new tentacles arise on either side of the median plane in the mid-distal (dorsal) region of the lophophore.

A narrow platform, or diaphragm, is present at the base of the tentacles; ventrally it is continuous with the large bilobed epistome; dorsally it is interrupted in the middle line where the new tentacles originate (see Text-fig. 5, p. 402). On the diaphragm is a ciliated tract, the vestibular groove, leading to the mouth. The ventral lip is considerably smaller than the epistome, and appears as a ciliated lobe between the bases of the two most ventral tentacles (Text-fig. 5, p. 402).

Normally in *Loxosoma* and *Pedicellina* the tentacles are extended, but when the animals are disturbed, or many distasteful particles are present in the water, the tentacles are bent inwards and folded away within the vestibule, while a delicate fold of skin, the velum or tentacular membrane, growing from the edge of the calyx at the bases of the tentacles, is drawn over the retracted tentacles by the contraction of a sphincter muscle present in its circular margin. The opening into the vestibule is thus reduced to a very small orifice. The appearance of *L. crassicauda* with tentacles withdrawn and lophophore closed is sketched in Text-fig. 2. The sphincter



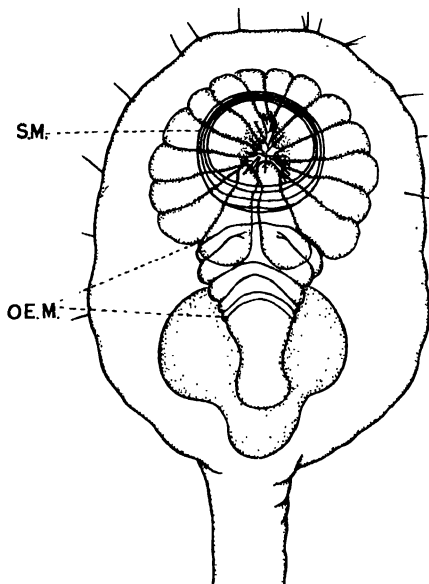
TEXT-FIG. 1.

L. crassicauda. Sketch of a living, unnarcotized animal to show the backward bending of the lophophore during feeding, the direction of the water currents set up by the lateral cilia of the tentacles, and of the rejection current (R.C.) caused by the epistomial cilia. $\times 70$.

is not as strongly developed in this species as in some, for instance, *L. singulare*, and in consequence the opening left into the vestibule is fairly large. Even in individuals killed

unnarcotized, the sphincter contracts little more than is shown in Text-fig. 2. In *L. crassicauda* the two most ventral tentacles—those on either side of the mouth—fold outside and across the adjacent tentacles.

The tentacles of *Loxosoma* and *Pedicellina* are



TEXT-FIG. 2.

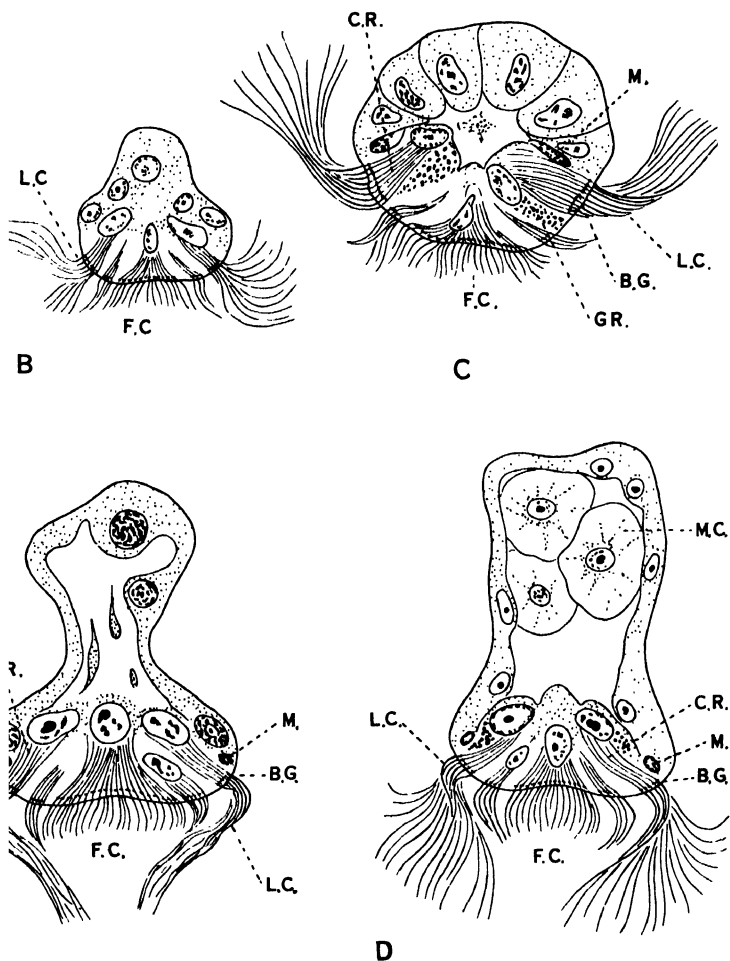
L. crassicauda. Ventral view of living animal with tentacles withdrawn into the vestibule, and lophophore closed. The only muscles shown are the sphincter of the lophophore (*S.M.*) and the muscles constricting the oesophagus (*O.E.M.*). Only the alimentary organs are shown. $\times 140$.

supplied with nerves; one nerve enters each tentacle and gives off branches to the sense-cells (Harmer 13, pp. 271, 273). In *L. crassicauda* the nerves are visible in the living animal. Muscle-fibres also enter the tentacles, and to these are due the movement of the tentacles during feeding. Independent bending movements of a tentacle—sideways, and inwards and outwards—are observable. To the contraction of these longitudinal muscles, together with the sphincter in the velum, is due the

folding away of the tentacles into the vestibular cavity, when the animal is disturbed. In *Loxosoma* and *Pedicellina* there appear to be two longitudinal muscles in each tentacle; these run close to the lateral ciliated cells. The fibres are not easily identified in transverse sections.

The tentacles of *Loxosoma* are roughly triangular in cross-section, with the base of the triangle facing the lophophoral space (Text-fig. 6, p. 403). In *L. obesum* the triangle is almost equal-sided (Text-fig. 3, b), in *L. crassicauda* elongated (Text-fig. 3, a), and in this latter species, especially the two long sides of the triangle, are somewhat concave. This concavity is not due entirely to shrinkage of the tentacles on fixation, for it is seen in the living animal. The tentacles of *Pedicellina cernua* tend to be roughly rectangular in cross-section (Text-fig. 3, d), except near the tips, where they are triangular.

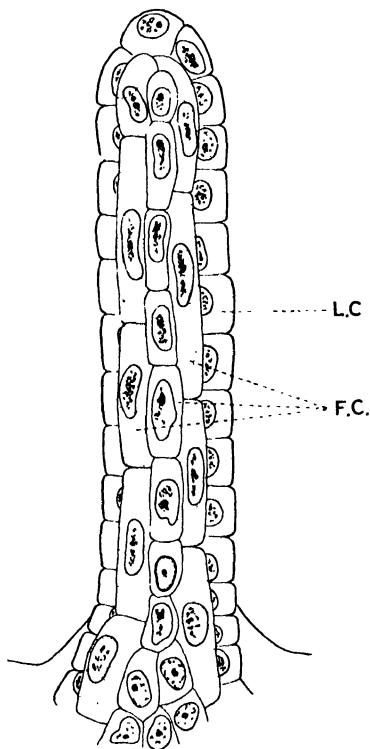
The epithelium of the tentacles consists of three kinds of cells: (1) ciliated cells; (2) non-ciliated cells; and (3) a few scattered sense-cells, bearing one or more stiff tactile hairs, which occur among the unciliated cells. The non-ciliated epithelium is found on the outer and lateral surfaces of the tentacles; there appears to be no regular arrangement of the cells: the nuclei are roundish. The cells on the inner or ciliated surface are in three tracts; a frontal (middle) and two lateral. The appearance in surface view of the ciliated cells of the tentacles of *L. crassicauda* is shown in Text-fig. 4. The frontal cells forming the middle tract are in three rows, and there is no interlocking of the cells. The middle row is slightly depressed to form a very shallow groove (see Text-fig. 3). The cells are rectangular in surface view, the two outer rows being especially elongated. The nuclei are long and narrow, and generally placed horizontally, but they may be twisted. The nuclei of the frontal cells, especially of those towards the bases of the tentacles, are frequently irregular in shape, as are also those of the cells forming the vestibular groove at the base of the tentacles. The frontal cells bear short cilia, those on the two outer rows being somewhat longer than those on the middle row (Text-fig. 3).



TEXT-FIG. 3.

Transverse sections of the tentacles of *Loxosoma* and *Pedicellina*. A. *L. crassicauda*. B, C. *L. obesum*. C, section towards the base of a tentacle. D. *Pedicellina cernua*. B.G., basal granules; C.R., ciliary rootlets; F.C., frontal cilia; GR., granules in lateral ciliated cells; L.C., lateral cilia; M., ? muscle-fibres; M.C., large cells in tentacle of *Pedicellina*. A and C, fixed in strong Flemming's fluid without acetic acid; B, Bouin's fixative; D, corrosive sublimate: all stained in Heidenhain's iron haematoxylin and acid fuchsin. \times ca. 1200.

The cells of the lateral series are almost cubical, and have large oval nuclei (Text-fig. 4). They each bear a single row of long cilia. On fixation each cilium separates into its constituent



TEXT-FIG. 4.

Inner surface of a tentacle of *L. crassicauda* showing the arrangement of the ciliated cells. *F.C.*, the three rows of frontal cells; *L.C.*, lateral cell-row. From an animal fixed in formalin, stained in borax carmine and picro-nigrosin, and mounted entire in alcoholic Canada balsam. $\times 735$.

fibres, and therefore in sections has the appearance of a tuft of fine cilia (Text-fig. 3).

Assheton (2, p. 136) described the cells of the ciliated surface of the tentacles of *L. saltans* as being in three rows, as did Kowalewsky (15, p. 3) in *L. neapolitanum*; Nickerson

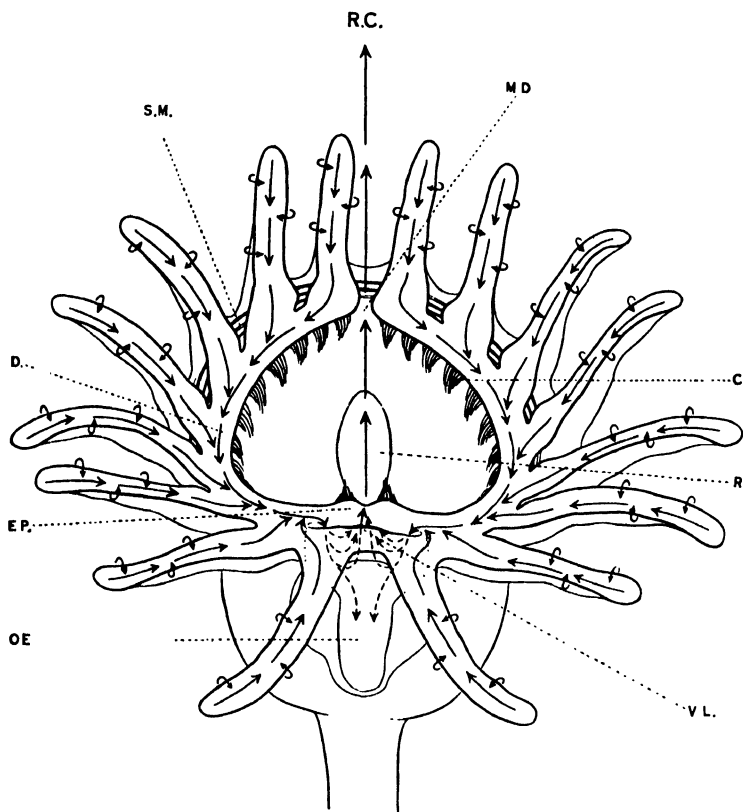
(19, p. 354) found no definite arrangement in *L. davenporti*. Kowalewsky says that the middle row is depressed to form a groove, but that the two lateral rows alone bear cilia; the latter statement is most probably incorrect, for while transverse sections of the tentacles of *L. crassicauda* and *L. obesum* show them to be very slightly grooved, the middle tract of cells also is ciliated. In figures of transverse sections of the tentacles of *L. saltans* (2, Pl. 7, fig. 18) and *L. davenporti* (19, Pl. xxxii, figs. 9 and 10) cilia uniform in length are shown over the whole of the inner surface, and it may be noted that a similar uniformity in the length of the cilia is shown by Cori (9, Text-fig. 5, p. 9) on the tentacles of *Pedicellina cernua*. An examination of living specimens of the species of *Loxosoma* found at Plymouth, and also of *Pedicellina cernua*, showed that while all the rows of cells on the inner face of the tentacles bear cilia, those of the middle rows are much shorter and finer than those on the two outer rows. An examination of the living tentacle is desirable, as sections do not always show clearly the difference in length of the frontal and lateral cilia. The cilia on the two outer rows of cells are very long, being about 35μ to 45μ long in *L. crassicauda*, and lash inwards (from the abfrontal to the frontal surface) across the length of the tentacles, except at the tip—occupied by a single cell—where they beat along the length. The short frontal cilia beat along the length of the tentacles and towards the base. In the living *L. crassicauda* the lateral cilia are seen to be in groups of about twelve to fifteen to a cell, separated by slight intervals corresponding to the cell-walls: the number of cilia may be counted in a cell which has worked out of the epithelium. The metachronal rhythm of these cilia is characteristic, but difficult to describe: viewed from the frontal or abfrontal surface the appearance is of a double row of dots—one of which is very close to the bases of the cilia and is not always visible when the tentacles are viewed from some positions—while, at more or less regular intervals, cilia are extended. The effect of the rows of dots is no doubt due to the bending of the cilia during the stroke. All the cilia arising from a single cell do not beat in the same phase: if, however, the animal has been long

narcotized, and the rate of beat is much reduced, there is a tendency for them to beat more or less in the same phase.

THE CILIARY FEEDING MECHANISM OF THE ENTOPROCTA.

An undisturbed *Loxosoma* in the act of collecting food particles has the tentacles well expanded. The degree of expansion of the tentacular crown, however, varies; under natural conditions the tentacles form a shallow funnel (Text-fig. 1), but when under the influence of a narcotic, e.g. stovaine, they may bend outwards so that an almost flat plate is produced, and in extreme instances, the tips of the tentacles may even bend downwards. Text-fig. 5, which is a sketch of a narcotized animal, shows the tentacular crown rather more widely open than it would be under normal conditions. In *Pedicellina* the tentacles generally seem to be curved slightly towards the lophophoral space, though when the animals are narcotized the tentacular crown may become as widely expanded as that of *Loxosoma* under similar conditions. While feeding, the calyx, with the lophophore, is turned in different directions. In *Loxosoma* (*L. crassicauda*), as previously mentioned, the dorsal half of the lophophore is generally bent backwards, so that it is almost at right angles to the calyx. During expulsion of faeces the backward bending movement of the lophophore is marked.

Water is drawn into the tentacular funnel by the action of the long lateral cilia on the tentacles. The action of these is so energetic that it may be seen to shake the tentacles. The current enters between the outstretched tentacles (see Text-fig. 6), and sets away in front of the animal (see Text-fig. 1, p. 395). When the tentacles are fully expanded the current is therefore roughly from the direction of the attached end of the animal towards the free end: this causes the free-swimming bud to move with the lophophore hindmost. Particles carried in suspension in the water passing between the tentacles are thrown by the lateral cilia on to their inner, or frontal, surface, and passed by the short frontal cilia towards the base, and into the ciliated, vestibular groove, which leads to the mouth (Text-fig. 5). The grooved tract is interrupted in the median dorsal line of the

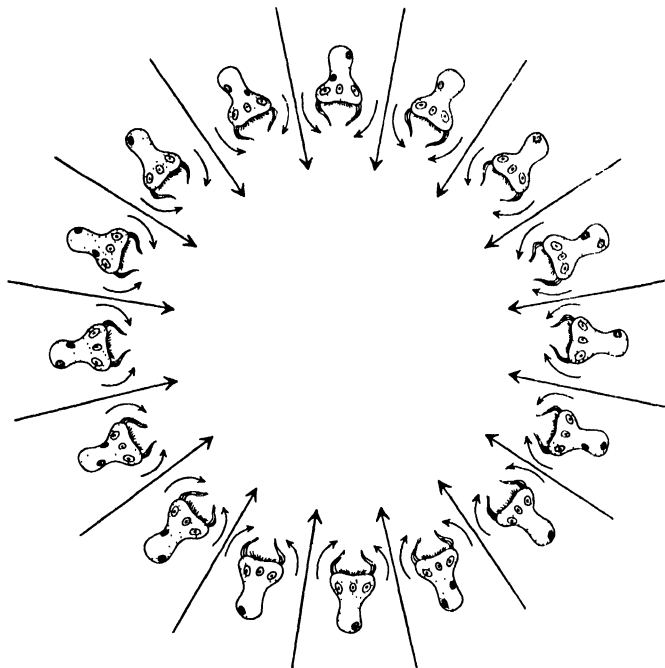


TEXT-FIG. 5.

Sketch of the tentacular crown of *L. crassicauda* showing the ciliary currents, and the direction of beat of the lateral cilia of the tentacles. Only the cilia (*C.*) arising from the edge of the diaphragm are shown. *D.*, diaphragm carrying ciliated vestibular groove; *EP.*, epistome; *M.D.*, mid-distal (dorsal) region of lophophore; *OE.*, oesophagus; *R.*, rectum; *R.C.*, rejection current set up by the cilia on the epistome; *S.M.*, sphincter muscle in the velum; *V.L.*, ventral lip of mouth. The tiny arrows show the direction of beat of the lateral cilia of the tentacles. $\times 140$.

lophophore, and particles travelling down the tentacles on either side of this point pass in opposite directions towards the mouth. The path followed by particles from the tentacles slopes in the direction of the mouth in passing into the vestibular

groove, except in the case of those from the tentacles on either side of the mouth, which follow a path sloping slightly away from it, and join the collected stream from the tentacles of its side, at the right and left corners of the mouth respectively (Text-fig. 5). During feeding, muscular movements of the



TEXT-FIG. 6.

Transverse section through the tentacular crown of *Loxosoma* (*L. crassicauda*) showing the direction of the water currents set up by the lateral cilia of the tentacles. The small arrows show the direction of beat of the lateral cilia. Somewhat diagrammatic. $\times 231.4$.

epistome, mouth region—including that part of the lophophore carrying the two most ventral tentacles—and oesophagus occur.

While feeding with expanded tentacular crown the animal may partly close it with a sudden clutching motion (the movement of the lateral cilia ceasing), and as rapidly extend the tentacles again (the ciliary beat recommencing): or one

tentacle may be flicked inwards, without the others being affected; this appears to occur when a particle—perhaps usually a free-swimming ciliate—strikes the outer surface of a tentacle, where the tactile hairs are found; even the presence of large particles on the inner surface does not appear to call forth this response. Occasionally a single tentacle is bent slowly inwards into the vestibule, and may remain in this position for some minutes, and then be slowly straightened.

L. crassicauda may occasionally add to its diet organisms too large and active to be captured in the usual manner. Small, actively swimming ciliates, which are too powerful swimmers to be carried by the water current of the *Loxosoma*, at times blunder within the circle of tentacles and penetrate into the vestibule. The *Loxosoma* immediately and rapidly approximates the tentacles, bunching them tightly together, and reducing the circumference of the lophophore. The ciliate is thus trapped within the vestibule, and it either accidentally, or helped by the activity of the cilia of the vestibular groove, reaches the mouth and is swallowed. Not till then does the *Loxosoma* expand its tentacles. One individual was observed to capture six ciliates, at intervals, in this manner, and others were seen behaving in a similar way, with two or three already in the stomach.

Particles may be accepted as food or rejected from one cause or another. When unwanted, unpleasant, or too numerous food particles are carried in the food current the animal may reject them in one of several ways, its behaviour seeming to be rather capricious. In extreme instances it rejects them in a most definite manner, by closing the lophophore and contracting violently. On other occasions the mouth may be closed, but more generally the particles are allowed to enter and are then rejected. In the latter case the upper part of the oesophagus is constricted by circular muscle-fibres (see Text-fig. 2, *O.E.M.*), and particles entering the right and left corners of the mouth are carried out again in two converging streams on to the oral surface of the epistome, from which they pass off between the two lobes in a median stream (see Text-fig. 5) to join the main water current setting away in front of the animal. The rejection

current (Text-figs. 1 and 5, *R.C.*) set up by the cilia on the epistome is easily distinguished in animals in which the lateral cilia of the tentacles are more or less motionless, and therefore the main water current almost in abeyance.

If the water were made thick with much powdered carmine or strings of large diatoms, the lophophore was observed, on occasions, to bring about rejection by partly closing—to about the position shown in **3**, Text-fig. 1, *b*—with most of the long lateral cilia of adjacent tentacles motionless, with their tips interlaced; thus while the main water current was very greatly reduced, the interlaced cilia would act as a filter. The frontal cilia and those of the vestibular groove, continued to beat, and particles already within the lophophoral space were being drawn on to the inner faces of the tentacles, thence into the vestibular groove, and so on to the epistome, continuously.

L. crassicauda was found to feed freely on *Nitzschia closterium* var. *minutissima*.¹ When, however, too great a quantity of the culture was added, the animals very soon, perhaps in a minute or two, had swallowed sufficient. They then, though continuing to hold the tentacles expanded, much reduced the supply of diatoms by keeping the lateral, water-current producing, cilia practically motionless wrapped across the inner surfaces of the tentacles. Those towards the bases of the tentacles were generally active, but those on the distal halves were either all motionless, or only an odd group of cilia here and there showed activity. The activity of the lateral cilia was fitful, numerous intermissions occurring, the tentacular crown being suddenly half closed and then slowly opened, these movements coinciding respectively with the stopping and the recommencement of the beat. Occasionally a single tentacle might be curved slowly inwards and downwards to the vestibule. During this time such diatoms as reached and entered the mouth were carried out and expelled from off the epistome. The behaviour described above has also been observed to occur for no apparent reason, in sea-water almost free of organisms, but there is a possibility that it might be due to the methods of observation.

¹ Dr. E. J. Allen, F.R.S., very kindly supplied the culture of diatoms.

A few *L. crassicauda*, with the stomach full, occasionally reacted to the presence of numerous diatoms (*Nitzschia*) in the water in a different way: the tips of the tentacles were approximated, the tentacles being bunched together, and the circumference of the lophophore reduced. They might remain like this for several minutes, then slowly expand the tentacles.

At other times when *L. crassicauda* was observed in water with numerous diatoms, after obtaining sufficient food, they kept the tentacles well expanded, with the lateral cilia especially active, and relied on the rejection current from the epistome to carry off the unwanted diatoms, in the manner previously described (see p. 404).

The Behaviour of the Lateral Cilia on the Tentacles of *Loxosoma crassicauda*.—While the frontal cilia of the tentacles, and those clothing the vestibular groove at the bases of the tentacles, beat continuously, the lateral cilia of the tentacles of a healthy *Loxosoma* frequently cease beating, and would appear to be under the nervous control of the animal. Intermission of the ciliary beat of the main water-current producing cilia also occurs in the Ectoproct Polyzoa, both in the fresh-water (see Nitsche on *Alcyonella fungosa* **21**, p. 27) and in the marine forms (see Borg **5**, p. 248), and in *Phoronis* (**11**, p. 163). *Phoronis hippocrepia* at Plymouth, though left undisturbed in their stone during observation, held the lateral cilia more or less motionless, and several attempts made to observe the animals feeding were unsuccessful.

A healthy *Loxosoma* while feeding frequently clutches all the tentacles inwards, while all the lateral cilia suddenly and simultaneously become motionless, held wrapped across the inner surface of the tentacles in the position of the end of the effective stroke. Such behaviour may occur in response to no perceptible stimulation, or may occur when large particles strike the tentacles, or when the tube of the microscope is gently tapped. If the tube is tapped sharply the animal retracts the tentacles entirely, closing the lophophore. After successive gentle tappings the animal becomes less sensitive, and sharper ones are needed to call forth the reaction.

If the stimulation has been slight the tentacles almost immediately begin to straighten out, and the cilia to start beating. The ciliary beat may begin while the tentacles are still curved, or not until they are practically fully extended.

The recommencement of the beat after a period of quiescence is not simultaneous on all the tentacles, or even on the same tentacle. A wave of activity passing over the cilia may begin at the base of the tentacle and travel rapidly towards the tip; this is perhaps the most usual behaviour. The two sides of a tentacle, however, appear to be independent, for the movement may not begin at the same moment, or travel at the same rate, on both sides. The recommencement of the beat does not invariably take the form of a wave of activity passing from the base to the tip of the tentacle. A slight variation is that the cilia on the first two or three cells of one side at the base may be late in starting. At other times the cilia on two or three cells at the tip may start beating first, followed by those on the basal part of the tentacle, but there is irregularity in the sequence in which the cilia on the different cells become active. During a series of gentle stimulations the cilia of a certain cell of a tentacle of one individual were consistently slow to begin beating after an intermission, and might be several seconds behind those on adjacent cells. It would seem that the cilia arising from a single cell generally become active more or less simultaneously, but it has been observed that the start of beat of the separate cilia may be independent; this is especially seen when the cilia of a cell lag considerably behind those on other cells in becoming active.

As previously mentioned, under certain conditions—for instance when numerous *Nitzschia* are present in the water and the animal has taken sufficient food—*L. crassicauda* reduces the water current by holding many of the cilia motionless. A certain number of cilia, however, are active, chiefly those on the basal halves of the tentacles springing from the ventral half of the lophophore. Such groups of cilia as are beating appear to be beating metachronically. The cilia on the distal halves of some of these tentacles may remain motionless, wrapped across the inner surface, during successive periods of

activity and quiescence of the cilia on the basal halves: they may remain motionless for as long as ten minutes, though this is probably unusual.

Although sudden inward bending movements, of all the tentacles together, appear to be invariably accompanied by the stoppage of the beat of the lateral cilia, slow bending movements of a single tentacle, even if the end enters the vestibule, are not so accompanied. The sudden flicking inwards of a single tentacle is unaccompanied by the stoppage of beat of the cilia on the others, or, I believe, on that concerned, though it is difficult to be certain of the latter, as, during the movement, the tentacle passes rapidly out of focus.

In *Loxosoma* the stoppage of beat of all the lateral cilia on all the tentacles is simultaneous, while the start of the beat of the group of cilia arising from a single cell, and perhaps even of separate cilia, is independent. Carter (7, p. 11) has found that in the nudibranch veliger the stoppage of beat of the velar cilia is simultaneous, but that the start of the beat of the separate cilia is independent, and he says, 'this difference between the behaviour at the beginning and end of the intermission suggests that the recommencement of the beat is due rather to the passing away of the impulse which caused the intermission than to a new impulse'. Whether this would be sufficient to explain the behaviour of the lateral cilia of *Loxosoma*, where a number of the lateral cilia on a tentacle may remain motionless for some minutes, while the remainder on the same tentacle experience successive periods of activity and quiescence, is perhaps doubtful.

In *Loxosoma* the long cilia on the single cell at the extreme tip of each tentacle, and which beat along its length, are frequently seen motionless, bent slightly in towards the inner surface; in side view they then have the appearance of a single very stout cilium. These cilia appear often to lag some time behind the others in becoming active after an intermission.

When the tentacles are withdrawn into the vestibule and the lophophore is closed (see Text-fig. 2, p. 396), the lateral cilia beat, but rather irregularly owing to the restricted space; intermissions occur, and during these the two rows of cilia are

seen wrapped across the inner surfaces of the tentacles, the tips of those of opposite side interlacing.

Under the influence of stovaine, the cilia beat without intermission, but there is a tendency, if the narcotic be used over a period of half an hour or more, for the ciliated cells to break away. Generally those towards the tips of the tentacles are shed first. Even under normal conditions there appears to be some tendency for the lateral ciliated cells gradually to work out of the epithelium, individuals being observed with cells in the process of being shed. Their place is possibly taken by new cells, and in this way worn-out ciliated epithelium renewed. It might be noted that Carter (7, p. 11) describes the breaking free of the cells bearing the velar cilia in unhealthy nudibranch veligers. Cilia on the isolated lateral cells of *Loxosoma* may continue to beat actively for a short time.

With ether, also, intermissions cease, but it seems that they reappear after a time. Contraction of the muscles, including those of the tentacles and lophophore were observed, both during the time intermissions were inhibited, and when they had returned. This drug, however, was only used two or three times and its action not fully studied. The animals during observation were placed in a solid watch-glass, covered with a sheet of glass, so as to prevent evaporation of the ether.

The occasional intermission of the lateral cilia in healthy individuals, together with the ceaseless beating when under the influence of a narcotic, is suggestive of the behaviour of the velar cilia of the nudibranch veliger under similar conditions (7), and it would appear not improbable that they are similarly under the nervous control of the animal. That the tentacles of *Loxosoma* are supplied with nerves has been demonstrated by Harmer in *L. crassicauda* (13): the nerves, however, were traced to the sense-cells present on the unciliated surface of the tentacles.

In *Loxosoma*, inhibitory control occurs of cilia concerned with feeding; so far as is known such control is almost restricted to locomotory cilia. According to Fedele, however, the branchial cilia of *Doliolum* are under the control of the animal (see Gray 12, p. 125).

A tufted fringe of cilia, 20 to 30 μ long, occurs along the free edge of the diaphragm, hanging down into the vestibule, and is continuous across the epistome, a 'tuft' occurring on either lobe (see Text-fig. 5, p. 402). These cilia appear to be frequently motionless, with the exception of the two groups on the lobes of the epistome; on the occasions when they have been observed lashing, they do so irregularly, a few groups (a group to a cell) beating, while the rest are motionless. They seem to be rather less inactive when the lophophore is closed. The beat is at right angles to the edge of the diaphragm, but the direction of the effective stroke was not determined. Their function remains obscure; it is possible that they may prevent particles—straying from the vestibular groove—from falling into the vestibule (particles are not seen within the vestibule of a healthy *Loxosoma*); or they may effect a circulation of water among embryos in the vestibule. These cilia would appear to belong to the type which is motionless, or only feebly active, except when a stimulus is applied (see Gray 12, p. 125). The stimulus which would set these cilia moving was not determined; particles passing round the vestibular groove did not necessarily cause them to become active. In a narcotized animal, in which the lateral cilia of the tentacles are beating rapidly and without intermission, the cilia hanging from the edge of the diaphragm are motionless, with the exception of the two groups on the lobes of the epistome, which may show some movement.

There appear to be no glands in connexion with the ciliated tracts on the tentacles, or with the vestibular groove; the large gland-cells which outline the vestibule parallel with the groove in *L. crassicauda* have been shown by Harmer (13) to open to the exterior.

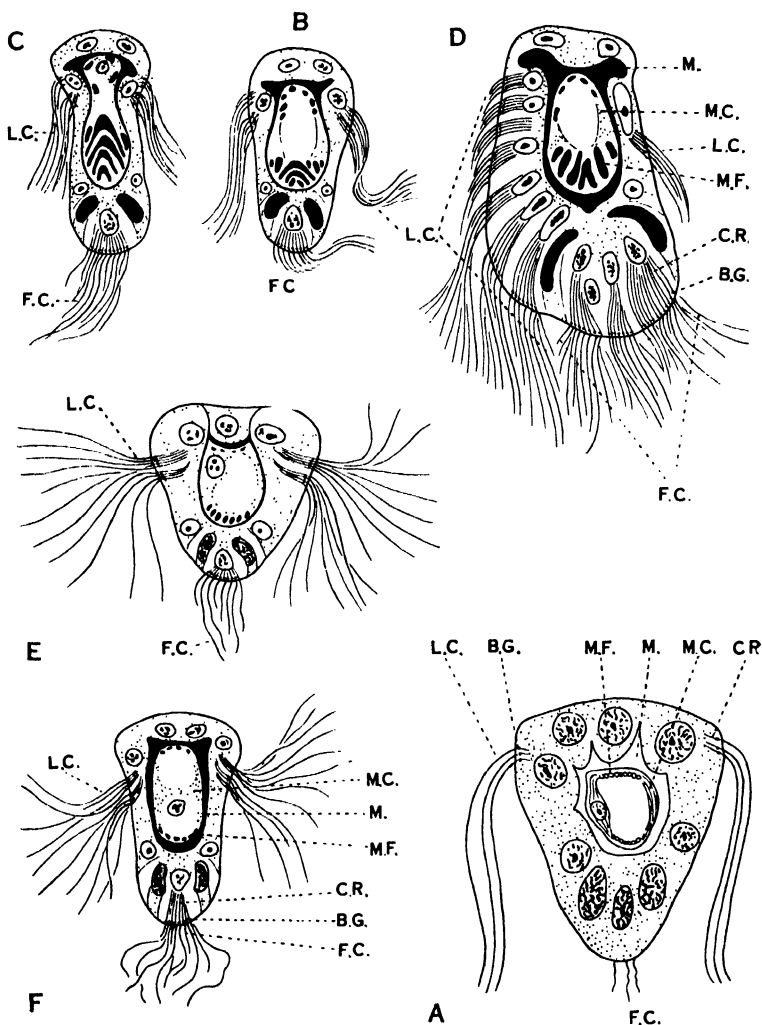
The ciliary feeding mechanism of *Pedicellina cernua* is essentially the same as that of *Loxosoma*. The direction of the ciliary currents on the tentacles and along the vestibular groove are shown by Cori (9) in his Text-fig. 3, p. 6, though he does not distinguish between the two kinds of cilia on the tentacles. The cilia are differentiated into lateral cilia (see Text-fig. 3, D, p. 398) beating across the length of the tentacles, with the effective beat from the abfrontal to the frontal surface.

and the frontal cilia beating along the length, from the tip towards the base. The metachronal wave of the lateral cilia of *Pedicellina* is of the same type as that of the lateral cilia of *Loxosoma*.

A RÉSUMÉ OF BORG'S WORK ON THE CILIARY FEEDING MECHANISM OF THE ECTOPROCTA, WITH A NOTE ON *FLUSTRELLA HISPIDA*.

A preliminary account of the ciliary feeding mechanism of the Ectoproct Polyzoa was published by Borg in 1923 (4), and a further one in 1926 (5). From his accounts it is evident that the method of feeding in the Ectoprocta is very different from that of the Entoprocta. Borg worked on the Cyclostomata, *Crisiella*, *Crisia*, *Tubulipora*, *Borenicca*, and *Lichenopora*, as well as several cheilostomatous and ctenostomatous species (5, p. 246): at Plymouth the feeding of *Flustrella hispida*, one of the Ctenostomata, was especially noticed.

The form of the tentacles in the Ectoprocta, as in *Loxosoma*, is more or less triangular in cross-section (Text-fig. 7), in some forms (*Crisiidae*), however, with the apex of the triangle truncated (5, p. 216), but while in *Loxosoma* the base of the triangle faces the lophophoral space, in the Ectoprocta the apex faces the space (cf. Text-figs. 6, p. 408, and 8, B). The outer surfaces of the tentacles are unciliated, but bear a number of very long, stiff, tactile hairs. The lateral cilia (see Text-fig. 7) occur near the abfrontal (outer) face of the tentacles (at either corner of the base of the triangle in cross-section), are long, and beat from the frontal to the abfrontal surface; these are the main water-current producing cilia. From transverse sections it would seem that the lateral cilia occur in a double row on either side of the tentacles. According to Borg these cilia do not beat at right angles to the length of the tentacle, but somewhat obliquely downwards, and the tip of each cilium traces out an elliptical path. They have a marked metachronal wave, which passes up one side of a tentacle and down the other, and runs, therefore, at right angles to the direction of beat of the cilia.



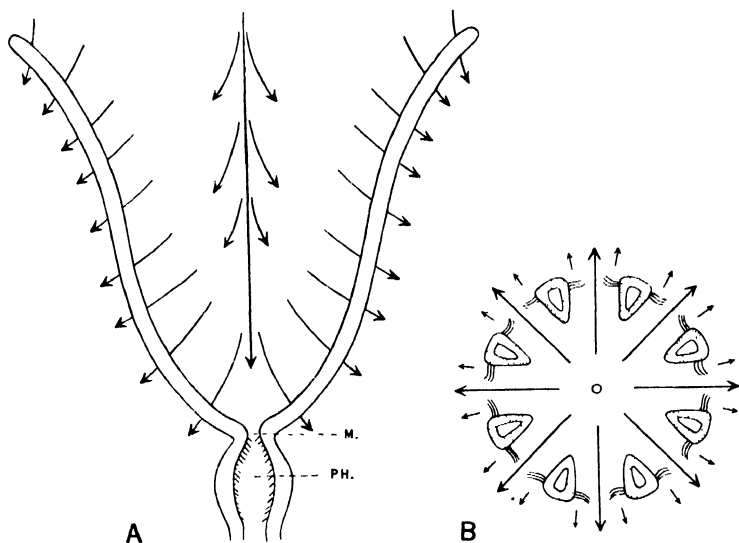
TEXT-FIG. 7.

Transverse sections of the tentacles of Ectoproct Polyzoa. A. *Lichenopora fimbriata* (Cyclotomata). (After Borg.) \times ca. 2000. B, C, D. *Flustrella hispida* (Ctenostomata). B, through distal region of tentacle; C, through basal region of tentacle; D, through base of a tentacle forming part of the rejection tract. \times ca. 1200. E, F. *Electra pilosa* (Cheilostomata). E, through distal region of tentacle; F, through basal region of tentacle. \times ca. 1470. B.G., basal granules; C.R., ciliary rootlets; F.C., frontal cilia; L.C., lateral cilia; M., homogeneous membrane; M.C., mesoderm; M.F., muscle-fibres. B-D, corrosive sublimate; E, F, Bouin's fixative; B-F, iron haematoxylin and acid fuchsin.

The development of the cilia along the frontal face would appear to vary widely in different forms.¹ They may be (a) absent, or Borg (5, p. 217) states, so feebly developed that he was unable to find them, as in most Cyclostomata; in others (b) short and thinly scattered as in the Crisiidae, and in some other forms, for instance, *Berenicea patina*, *Diplosolen obelia*, and *Lichenopora fimbriata* (Text-fig. 7, A) (5, p. 216); or (c) fairly numerous and long as in *Flustrella hispida* (Text-fig. 7, B-D) and *Aleyonidium* (Ctenostomata). Also in *Electra pilosa*, one of the Cheilostomata, the frontal cilia are fairly long, at least towards the base of the tentacles, though perhaps not very thickly set (Text-fig. 7, E and F). Marcus (see Borg, 5, p. 248) says of the frontal cilia of *Marrella repens* that they are immovable and stiff. In the first instance (a) a frontal current along the length of the tentacle is obviously absent; in the second instance (b), Borg (5, p. 248) says he has occasionally seen a particle, which has stuck to one of these cilia, moving slowly along the frontal face towards the mouth, but that these frontal cilia play quite a subordinate part in the nutrition; in (c) *Flustrella hispida*, where the frontal cilia are well developed, they approach the lateral cilia in length and there seems to be little or no movement of particles over them, except perhaps towards the lower part of the tentacular funnel. Here particles may occasionally be seen travelling down them into the mouth. Towards the base of the lophophore, where the tentacles are crowded together, the frontal cilia are especially long, while the laterals appear somewhat reduced in length. The chief function of the frontal cilia—especially of those towards the bases of the tentacles—in *Flustrella hispida* would appear to be to help produce and direct the main water current towards the mouth.

¹ It is possible that the frontal cilia are longer than they appear in transverse sections. As these cilia beat along the length of the tentacles, it is possible that in preserved material they may lie at an angle to the frontal surface, and in transverse sections would be cut across. The lateral cilia, on the other hand, beating across the length of the tentacles, would be seen at their full length in transverse sections.

In the *Ectoprocta* the extended tentacles form a funnel with the mouth at the base: in *Flustrella hispida* the shape of the lophophore is bell-like, the tips of the tentacles being bent outwards. Briefly the method of feeding as observed by Borg (5, p. 247) is as follows: a water current is produced by



TEXT-FIG. 8.

- A. Diagram showing longitudinal section through tentacular crown, mouth (*M.*), and pharynx (*PH.*), in *Cyclostomata*. B. Diagram showing section through tentacular crown. The arrows indicate the direction of water currents caused by movements of cilia. (After Borg.)

the lateral cilia of the tentacles, between which it passes outwards (Text-fig. 8) (that is in the opposite direction to the water current in *Loxosoma*) incidentally carrying with it many food particles. This results in the formation of a current directed straight down the lophophore to the mouth (Text-fig. 8, A). The muscular pharynx acts as a suction-pump which receives the food, and its effect is increased through the strong cilia of the epithelium of the pharynx, which move from above downwards. As Borg points out, the feeding mechanism cannot be regarded as very perfect, many particles escaping with the

water current passing out between the tentacles, and mostly only those in, or near, the median line of the lophophore reaching the mouth. Borg (5, p. 248) details the various means by which the animal increases the number of particles brought to the mouth, chief of which are the turning of the tentacular crown in different directions, and the alteration in the direction of the water current by the spreading and the contracting of the tentacles.

The methods resorted to by the Ectoprocta to prevent distasteful particles from reaching the mouth are, according to Borg (5, pp. 248, 249):

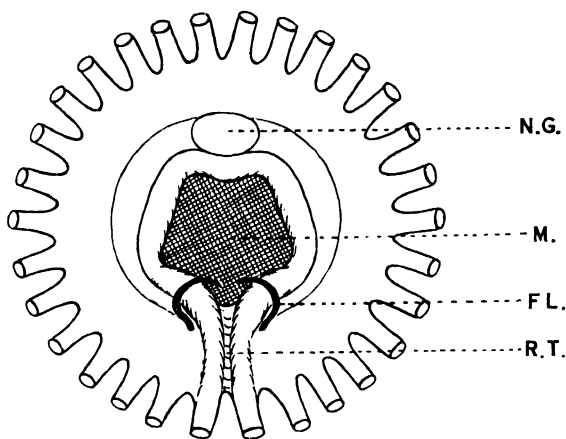
1. Complete or partial retraction of the tentacular crown.
2. Approximation of the tips of the tentacles, thus preventing the formation of the water current towards the mouth, while particles are whirled out between the tentacles.
3. Quick movement towards the median line of a tentacle to free itself from a useless particle, which has adhered to it.

The rejection of useless particles, which have already gained the region of the mouth, is carried out in the following different ways (see Borg, 5, pp. 249, 250):

1. The mouth remains closed, and particles are then usually carried away by the water streaming out between the bases of the tentacles.
2. 'Particles that have already been swallowed can again be ejected out of the stomodaeum, through a momentary alteration of the direction of movement of the cilia of the pharynx, and a quick opening and closing of the mouth.'
3. Ejected particles, and others too large to pass through the narrow spaces between the proximal parts of the tentacles, often collect in a little heap by the side of the mouth. When this occurs the animal first widens the tentacular crown, and then contracts it with great rapidity; in this way water at first streams in between the tentacles and then is forced out through the opening of the tentacular funnel, carrying with it the heap of particles.

The Method of Rejection of Unwanted Particles in *Flustrella hispida*.—The method of rejection of un-

wanted particles from the mouth region in *Flustrella hispida* is more specialized than is that of the forms described by Borg. It was noticed that in *Flustrella* if the animal does not wish to feed, particles passing into the pharynx travel out again at a certain point ventrally, and, passing between the bases of two tentacles, are carried away in the main current

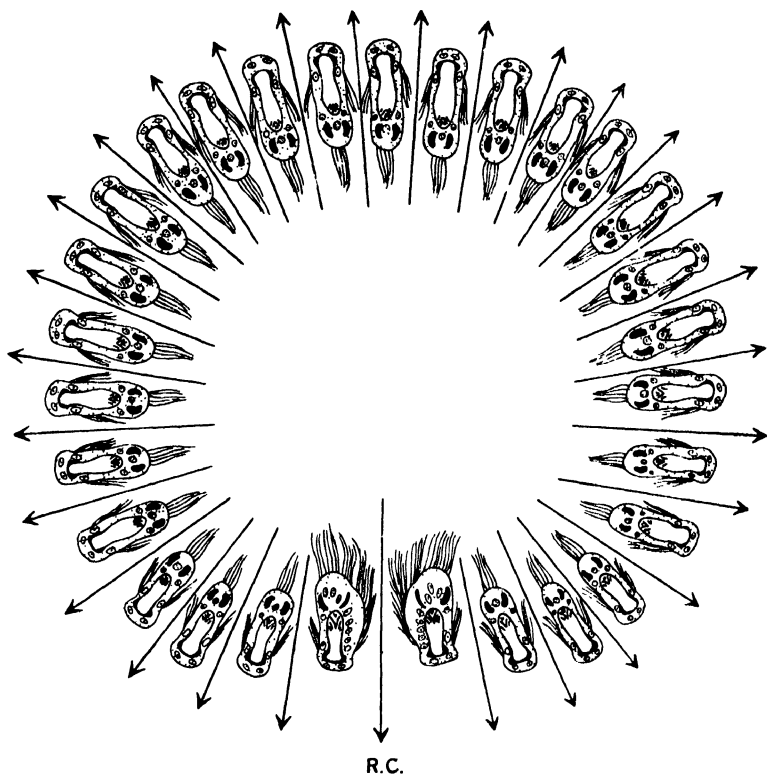


TEXT-FIG. 9.

Surface view of the lophophore of *Flustrella hispida*. (Slightly modified after Prouho.) *FL.*, flagellum; *M.*, mouth; *N.G.*, nerve ganglion; *R.T.*, ciliated rejection tract. $\times 225$.

setting away from the animal. Closer observation showed that there is a narrow ciliated rejection tract in this region, leading from the mouth outwards between the bases of the two tentacles (Text-fig. 9). These form part of the rejection tract, the cilia for a short distance beating towards the tips. This tract is a continuation of a ventral groove in the pharynx (Text-fig. 12, p. 419) along which the cilia beat outwards towards the mouth. Looking down on an expanded lophophore, this region, with the tentacles on either side of it, can easily be distinguished (Text-fig. 9); its position can be determined in transverse sections through the base of the tentacular crown, owing to the larger size of the bases of the two tentacles forming part of the rejection tract (see Text-fig. 10). On either side of the groove is a large

flagellum (see Prouho, **24**, p. 564), or I am inclined to think a short, almost transverse, row of stout cilia (Text-fig. 11, *FL.*), which beat transversely towards the groove. Their position at the



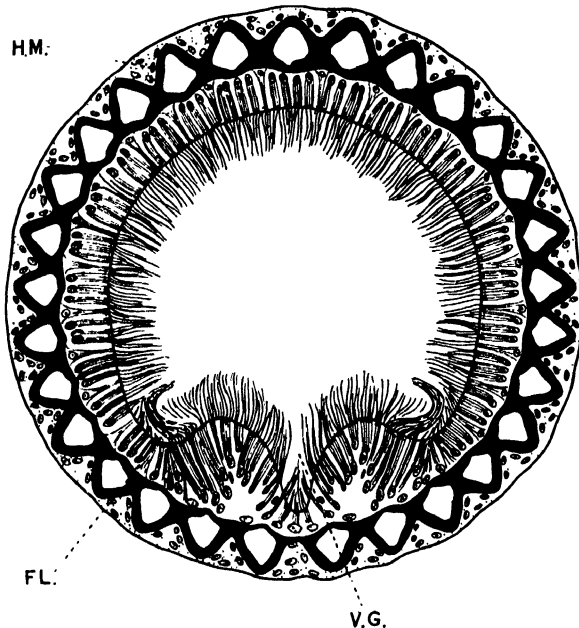
TEXT-FIG. 10.

Flustrella hispida. Transverse section through the tentacular crown (towards the base) showing the direction of the water currents set up by the lateral cilia of the tentacles. *R.C.*, arrow indicating the position and direction of the current carrying particles rejected from the pharynx; the bases of the tentacles on either side form part of the rejection tract. Somewhat diagrammatic. $\times 430$.

end of the beat into the groove is shown in Text-fig. 9: they do not beat continuously, but at irregular intervals.

In *Flustrella*, therefore, although the cilia clothing the

walls of the pharynx beat mainly in a downward direction, there is a groove in the mid-ventral line (Text-fig. 12) along which the cilia beat upwards, thus the passing of particles, and of water, out of the pharynx is not due to a momentary alteration of the direction of movement of the cilia of the pharynx, such as Borg (5, p. 249) found in the species he investigated. It is probable that muscular movement of the walls of the pharynx



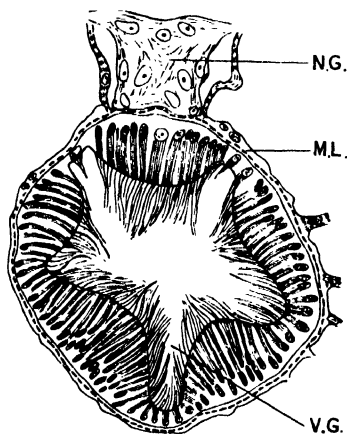
TEXT-FIG. 11.

Flustrella hispida. Transverse section through the lophophore at the level of fusion of the tentacles. *FL.*, flagellum (or short row of stout cilia?); *H.M.*, homogeneous membrane of the tentacles; *V.G.*, ventral groove or rejection tract. Corrosive sublimate; iron haematoxylin and acid fuchsin. Somewhat diagrammatic. $\times 430$.

determine whether particles be brought in contact with the outgoing tract of cilia, for during feeding the walls of the pharynx are in constant movement. Particles accepted as food collect, before being swallowed, in the region where the pharynx passes

into the unciliated oesophagus, but even from here there may be some slight loss of particles, as the ventral ciliated groove is continued for a very short distance among the unciliated epithelium of the oesophagus.

The peculiarity in the form of the buccal region in *Flustrella*, and an allied genus *Pherusa*, was noted by Prouho (24, p. 564) in 1892, and he says, "La symétrie bilatérale du



TEXT-FIG. 12.

Flustrella hispida. Transverse section through the pharynx at the level of the nerve ganglion. *M.L.*, muscle-layer; *N.G.*, nerve ganglion; *V.G.*, ventral groove or rejection tract. Corrosive sublimate; iron haematoxylin and acid fuchsin. $\times 430$.

lophophore est ici rendue manifeste par cette disposition particulière qui, sans doute, doit être de quelque utilité à l'animal pour le choix ou la préhension de sa nourriture."

DISCUSSION.

From what is known of the structure of the tentacles and their ciliation in the fresh-water Polyzoa, it is probable that the ciliary feeding mechanism of this group is somewhat similar to that of the marine *Ectoprocta*, though no doubt modified owing to the horseshoe shape of the lophophore in the majority

of forms, and the presence of a large epistome. The lateral cilia in the Phylactolacmata have been described by Allman (1, p. 20), as beating towards the tips of the tentacle on one side and towards the base on the other, but he was apparently misled by a marked metachronal wave of the type of the lateral cilia of the marine Ectoprocta, and, as pointed out by Nitsche (21, p. 26), in reality the cilia beat across the tentacles (see also Kraepelin, in Borg 5, p. 245). It is very probable that the effective beat is from the frontal to the abfrontal face as in the marine forms. It might be noted that Gilechrist (11, footnote, p. 163) also alludes to the lateral cilia of the tentacles of Polyzoa as beating in opposite directions on each side of the tentacles.

It is of interest that a similar type of metachronal wave, that is one running at right angles to the direction of beat of the cilia, and in opposite directions on opposite sides of a tentacle, filament, or gill-bar, is found for the lateral cilia in widely different groups of animals in which these cilia beat in the same direction, namely, from the frontal to the abfrontal surface, and where their function is that of producing a water current. Groups of animals in which the lateral cilia have a rhythm of this type are: Ectoproct Polyzoa, Phoronis, Lamellibranchs, those Gastropods in which the gills are formed of distinct filaments, and the cilia are differentiated into laterals and frontals (i.e. Gastropods exclusive of Tectibranchs and Nudibranchs), Ascidians and Amphioxus.

It will be evident from the foregoing account that the method of feeding in the Entoproct and Ectoproct Polyzoa is very different; not only are the main water currents in the two groups in opposite directions (cf. Text-figs. 1, p. 395, and 8, p. 414)—illustrated by the fact that in *Loxosoma* a free bud, or detached small adult, swims with the calyx hindmost, while the opposite occurs in the Ectoprocta—but while ciliary currents (as distinct from water currents)¹ play an important part in the method of feeding in the Entoprocta,

¹ A clear statement of the distinctions between water currents and ciliary currents is given by Graham ('Trans. Roy. Soc. Edin.', vol. lvi, Part III, no. 29, p. 738, 1931).

they may be absent, or little developed, on the tentacles of the Ectoprocta. The difference in the method of feeding is reflected in the size of the particles taken in the two groups, the Entoprocta being restricted on the whole to finer particles than are the Ectoprocta.

The method of feeding in the Entoprocta is rather similar to that of *Sabella pavonina* as described by Nicol (20), though on a simpler plan, and without the specialized sorting mechanism of the worm. In *Sabella* the beat of the long cilia which maintain the main water current is also from the abfrontal to the frontal surface of the filaments, and they have a metachronal rhythm similar to that of the lateral cilia of *Loxosoma*. It might be noted, however, that while in *Sabella* these cilia when at rest have a marked S-form, those of *Loxosoma* are only slightly curved inwards.

The cilia producing the main water current in *Loxosoma* and *Pedicellina* are in the same position in regard to the frontal cilia, that is adjacent to them on either side, as in *Sabella pavonina* and certain other Cryptocephalous Polychaetes (see Nicol 20), and incidentally as on the dorsal filaments of Brachiopods (see Orton 23, p. 293)—though in the latter group the effective beat is in the reverse direction—and have been termed by Nicol latero-frontal cilia. While these cilia are undoubtedly latero-frontal in position, this term is perhaps not altogether advisable as it has been previously applied to, and has come to denote in particular, the slow beating, straining (see Gray¹ 12, p. 145, and Orton 22, p. 466)—and not water-current producing—cilia of the Lamellibranch Mollusca. As the long cilia on the tentacles of the Entoprocta agree in their function of producing the main water current—though the effective beat is in the opposite direction—with the lateral cilia on the tentacles of the Ectoproct Polyzoa, and incidentally with those of *Phoronis*, Lamellibranchs, certain Gastropods, Ascidians, and *Amphioxus*, they have been termed lateral cilia in this paper.

The ciliary feeding mechanism of the Ectoprocta would

¹ Gray (12, p. 145) also says that 'they appear to keep individual filaments apart, so giving freedom of action to the lateral cilia'.

appear to differ considerably from that of any group so far described in any detail, in that ciliary currents, if not absent, play a subordinate part, while the chief role is played by the water current—set up by the lateral cilia—in conjunction with a suction pump formed by the muscular pharynx.

Due acknowledgements have been made in the previous paper on 'The Loxosomatidae of the Plymouth Area'; in addition I wish to express my sincere thanks to Prof. J. H. Orton for reading the manuscript of this paper.

SUMMARY.

An account is given of the ciliary feeding mechanism of the Entoproct Polyzoa, and of the structure of the lophophore and tentacles. The long lateral cilia cause a current of water to pass inwards between the tentacles, and throw particles on to the short frontal cilia of the inner surface, which carry them to the vestibular groove leading to the mouth.

The behaviour of the lateral cilia of the tentacles of *L. crassicauda* is described, and it is suggested that they are under the nervous control of the animal.

A résumé of Borg's work on the ciliary feeding mechanism of the Ectoprocta is given, a note on *Flustrella hispida* being added. It is pointed out that the method of feeding in this group differs widely from that of the Entoprocta.

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The Development of the Amphibian Kidney.

PART II.

THE DEVELOPMENT OF THE KIDNEY OF TRITON VULGARIS AND A COMPARISON OF THIS FORM WITH RANA TEMPORARIA.

By

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Lecturer in Vertebrate Embryology in the Department of Zoology
of the University of Edinburgh.

With Plates 22 to 26 and 6 Text-figures.

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MATERIAL AND TECHNIQUE.

THE early larvae used in this investigation were bred from eggs laid in the laboratory; a certain number of metamorphic, and post-metamorphic, stages were obtained by field collection. The relation between length and age was found to be so variable that no useful result could be obtained by classifying the larvae before sectioning. The larvae used for the illustration of this paper are referred to their approximate ages, but it is quite impossible to quote any external characters which are accurately correlated with the degree of kidney development, it being only possible to gauge this latter from an examination of serial sections.

The heavily yolked stages were fixed in Smith's formol-bichromate-acetic mixture and stored in 5 per cent. formaldehyde; the replacement of alcohol by acetone in all subsequent manipulations was found to give a very definite improvement in the behaviour of the yolk under the microtome knife; all other material was fixed in Bouin's picro-formol-acetic. Embedding, after dehydration in either alcohol or acetone and clearing in oil of cedar, was carried out in 56° paraffin wax and 10 μ sections obtained through the usual planes. The sections were stained on the slide in Delafield's haematoxylin; differentiated in 3 per cent. hydrochloric acid in 70 per cent. alcohol and graded up to absolute alcohol, the stain being fixed and intensified at this point by exposure to ammonia vapour. Those slides, subsequently mounted in Gurr's neutral balsam, have as yet shown no signs of deterioration.

The reconstruction of kidney units from these series presented a problem of unusual difficulty, since it was found that no useful results could be obtained at a magnification of less than one thousand. This at once rendered impossible any idea of plastic reconstruction, since it is as impracticable to use wax plates of 10 mm. thickness, as it is to cut and mount a series of 1 μ or 2 μ sections from an object sometimes extending over 20 mm. Graphic reconstructional methods, however, are by no means simply applied at this magnification, for a visual enlargement of 250 or more does not embrace a sufficient

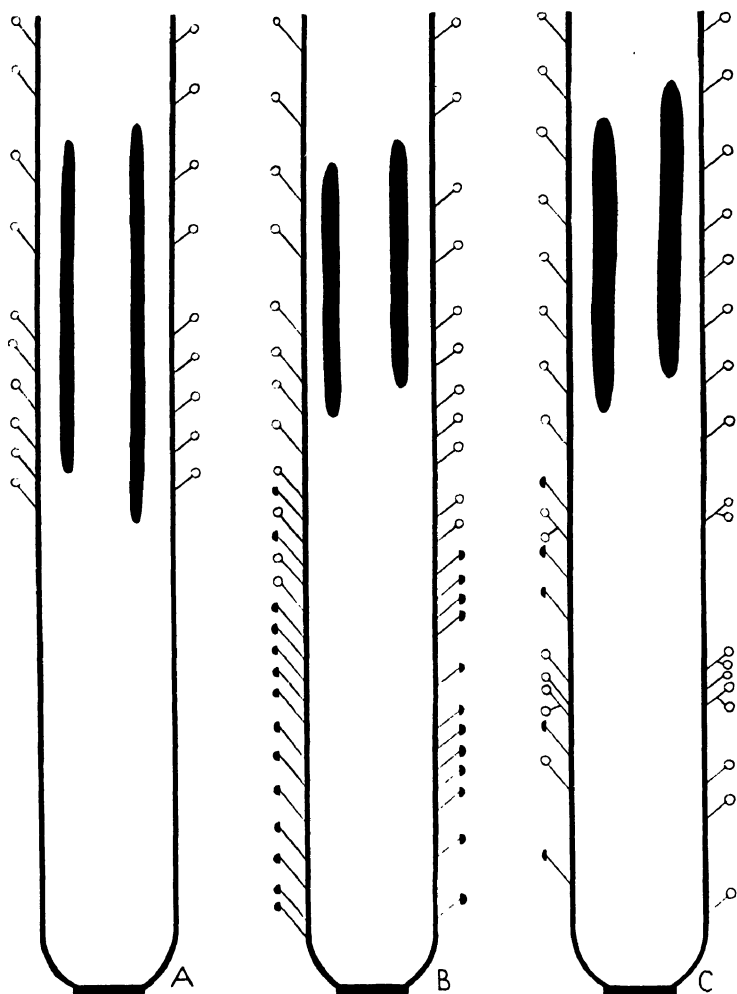
sectional area of the developing unit to permit of an accurate mental image of the tubules to be carried from one section to the next. A special technique was, therefore, evolved to meet these difficulties.

Three successive junctions of tubules with the archinephric duct were first identified and camera lucida drawings, at a magnification of about 100, made of every section between the first and last junction; these sections obviously covered all the tubules associated with the central junction. Each individual tubule was next traced throughout the drawings, starting from some easily identified point, such as the junction of the neck with the malpighian capsule, the different tubules being distinguished in different colours. From these marked drawings a small skeleton reconstruction was prepared along ordinary graphic lines with the aid of the actual sections. In these skeleton reconstructions each tubule was represented by a single line joining imaginary points placed uniformly down the centre of its lumen.

By this means, three separate cross checks upon the reconstruction were obtained; the actual sections, the camera lucida drawings and the skeleton reconstruction. The final reconstruction was then prepared with the aid of the skeleton reconstruction and the actual sections, any doubtful point being settled by reference to the drawings; the most useful centre line was found to be the centre of the dorsal aorta. This accurate reconstruction was then transferred, by means of a camera lucida, to a suitable paper and shaded, with the aid of the actual sections, to stimulate the correct degree of relief. Even though this method be slow and cumbersome, it appears to the present writer to be the only means by which high-power reconstructions of the complexity of that shown in Pl. 25 may be obtained.

GENERAL DEVELOPMENT.

The best method of gathering a general impression of the development of the kidney is by the consideration of a few, widely spaced stages; these are shown in Text-fig. 1. The examples here shown are diagrammatic reconstructions of the condition of the kidney units in: A, a larva about a week after



TEXT-FIG. 1.

Diagrammatic representation of the composition of the kidney at various stages. The vertical lines represent the archinephric ducts and the heavy black masses the gonads. Functional units are shown as white circles, undeveloped units as black semi-circles. A, week after hatching; B, intermediate between A and C; C, about to metamorphose.

hatching; c, a larva about to metamorphose; B, a condition about half-way between A and c. Each is based upon several examples and fairly represents an average condition at the ages specified. The parallel vertical lines represent the archinephric ducts entering the rectum (black rectangle) at their base, while the heavy, black, cigar-shaped areas show the extent of the genital strand or developing gonad at each stage. The individual kidney units are divided into two kinds. Those which are obviously in a functional condition, that is those which are furnished with a well-developed malpighian glomerulus, an open peritoneal funnel and connexion with the archinephric duct, are represented by white circles. Those in which the peritoneal funnel is not yet open, or in which the lumen of the connexion with the archinephric duct is still indistinct, are shown as black semi-circles. A comparison between fig. 5, Pl. 22, and fig. 31, Pl. 26, will give a very fair idea of this distinction in the earlier stages. Let us now see how the make-up of each kidney differs at this stage.

In the first stage there is a clear set of uniformly (not segmentally) spaced units in the sexual region, before any units have appeared in the hind region. These anterior units continue unchanged throughout the series and obviously represent the sexual kidney of the adult.

In the next stage (B) we find that a large number of units appear in the posterior region and that these units are neither segmentally, nor symmetrically, placed; there are, in fact, eighteen upon one side and thirteen upon the other, these figures being taken from an actual example. A few of the most anteriorly placed of these units have reached a sufficiently high degree of organization to be represented by a complete circle; these presumably belong to that region of the adult kidney in which the sexual and excretory units become confused. So far, then, we have no more than we might expect to find, except, perhaps, the unusually rapid development of the sexual region. When, however, stage c is examined there immediately appears the extraordinary fact that there are less units (of any kind) in the posterior region than there were in the previous stage, while there is also a quite remarkable lack of uniformity in that

undeveloped, developed, and secondarily attached units all appear at the same time. It should be explained at this point that these later reconstructions were obtained by plotting open peritoneal funnels against connexions with the archinephric duct; if, therefore, both these features had degenerated, the unit would be omitted from the reconstruction.

Are we then to believe that a certain number of these early mesonephric units have degenerated completely, thus carrying out a thinning process which leaves only a few mesonephric units to continue development? This theory, unfortunately, fails to account for the fact that certain almost undeveloped units still exist at this stage; it seems far more probable that these later units, were they mesonephric, would never have developed, rather than that certain of the existing units should have degenerated.

The alternative theory is that the whole set of early units has degenerated in the hinder region and is now in course of being replaced by a later set of units, from which will be derived the definitive kidney. It is this theory which most satisfactorily covers such new facts as the present writer has brought to light, and he is prepared to state that in *Triton* there are two quite distinct sets of units developed.

1. The true mesonephric units which, in the anterior region, continue through to the adult as the sexual kidney. In the posterior region, however, these units never acquire a clear and distinct connexion with the archinephric duct and finally degenerate.

2. A later or delayed set of posterior units which give rise, by a process later to be described, to the definitive kidney. These units overlap, in a time series, the original mesonephric units.

The facts upon which these statements are based will be discussed in detail in the following pages.

DEVELOPMENT OF THE MESONEPHROS.

1. *Origin.*—The origin of the mesonephros is, of course, from the intermediate mesoderm and appears closely to follow the course of production described by Hall (1904) for *Amblystoma*; that is, there is an early separation of an archinephric

duct and a later separation of a solid rod of blastema tissue which comes to lie along the outer side of the archinephric duct. This is seen on fig. 15, Pl. 24, where *ad* is the archinephric duct and *bl* the blastema. The whole of the available blastema appears to be condensed into this rod and there is no loosely bound tract of blastema tissue as in *Rana* (Gray, 1930).

This rod of blastema then breaks up into a number of spherical or oval vesicles, arranged at irregular intervals along the course of the archinephric ducts. There seems to be no reason to doubt that they are homologous with the corresponding vesicles in *Rana* (Gray, loc. cit.) save that in the latter form they are condensations in the blastema tract, whereas in *Triton* they represent the whole extent of the blastema. It must be remembered that in this latter form there is no reserve of blastema for the production of later units, and it therefore follows that, even though there be two distinct sets of units developed in the posterior region, the separate rudiments of both sets must be in existence from the very beginning.

2. General.—These spherical condensations of the blastema, which are quite obviously true nephroblast vesicles, develop differently according to whether they are:

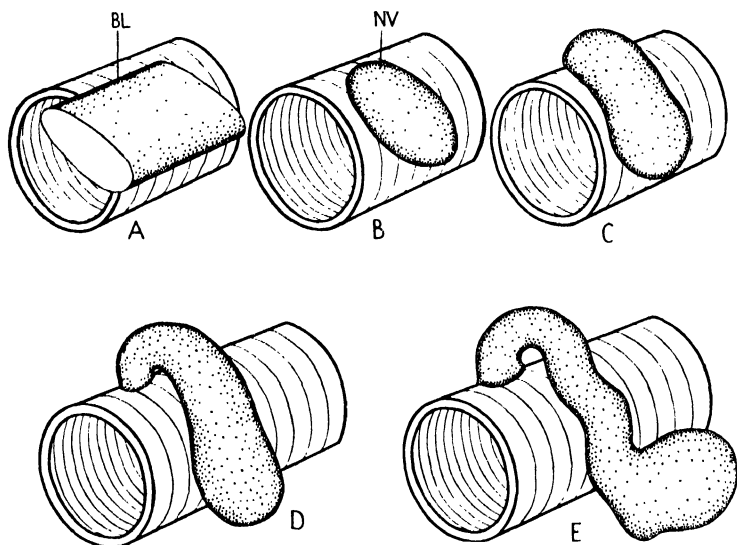
- (i) Rudimentary sexual units.
- (ii) Rudimentary posterior mesonephric units.
- (iii) Rudimentary definitive units.

These three types of vesicle cannot morphologically be distinguished one from the other; it is only in the course of development that differences become apparent. These differences, as will be later shown, depend not only upon the type of unit which is developed but also upon the period and rate at which development takes place. The anterior seven or eight units, lying in the region of the gonadic strands, develop rapidly to a functional condition. The posterior units develop in two series: an early series from which are derived the abortive mesonephric units, and a later series which give rise to the definitive kidney of the adult.

These three types of unit are quite distinct in the highest form which they attain, but develop in their primary stages along similar lines. The description which follows deals with

these primary stages and is here inserted to avoid needless repetition.

The nephroblast vesicle, composed of from twenty to thirty cells, elongates along a curve approximately following the cir-



TEXT-FIG. 2.

Series of diagrams representing the course of development common to all types of unit. *BL*, blastema; *NV*, nephroblast vesicle.

cumference of the archinephric duct to produce the form shown in Text-fig. 2B. This oval mass of cells now develops a lumen, apparently by splitting down its central region. The cells of each end then begin to divide and multiply far more rapidly than those of the central portion; the rate of division, however, is not equal at each end. Those of the dorsal end grow and curve downwards until they become pressed against the dorsal surface of the archinephric duct, with whose wall they fuse at this point. The lumen, however, does not continue into that of the archinephric duct, being still confined to the middle portion of the vesicle; the growth of the cells at this end, in fact, is that of a solid block, and not of the walls of a tubule.

While the attachment to the archinephric duct is being formed the cells at the ventral end of the oval rudiment have been increasing very rapidly in number, this increase, however, being directed to the thickening, rather than to the elongation, of the rudiment. We are thus left with the condition shown in Text-fig. 2 c. The lumen of the middle portion then extends into this region, so that the whole rudiment at this stage might be compared to a gourd or hollow-headed club.

The central portion now begins to elongate (Text-fig. 2 d), the rapid and irregular divisions of the cells tending to begin two bends, one directed anteriorly and the other posteriorly, along the course of the archinephric duct. These are the beginnings of that primary S bend which is presumably analogous to Henle's loop of the higher forms. The first beginnings of these bends are seen in Text-fig. 2 e.

So far we have dealt only with the stalk and attachment of the developing unit and have ignored the swollen head which is later to form the malpighian capsule and its attendant peritoneal funnel. The cells of the club-shaped tip divide, so far as one can make out, almost entirely along tangential planes, so that a thin-walled capsule is produced. The unit in this stage may be seen in figs. 1 and 2, Pl. 22. This unit, although a sexual unit already differing slightly from the normal in a manner explained in a later section, still clearly shows those features which have just been described. In fig. 1 the arrow (*a*) indicates the point of attachment to the wall of the archinephric duct (*ad*), and it will be noticed that the portion of the stem which lies in this region is, as yet, without a lumen. The X, seen in both figs. 1 and 2, lies in the cavity of the developing malpighian capsule (indicated by the letters *cmc*), whose ventral and distal walls in fig. 1, and the whole of whose wall as seen in fig. 2, are composed of a single layer of cells. In fig. 2, the anterior bend of Henle's loop (*hl*) lies within the plane of the section and has, at this stage, a quite distinct lumen.

Up to this point there is very little morphological difference between units examined from different parts of the body; there is, however, considerable difference in the period of development of these units as correlated with the general condition of external

development. Those in the sexual region will reach the stage described within a week or so after the embryo is hatched, while those in the posterior region will only have reached this condition about a month later. We will, nevertheless, now pass to a consideration of the further development, and ultimate fate, of these posterior mesonephric units, continuing the description as if there were no time lag between the different regions; this course is adopted since the development is less complicated than that of the sexual, or anterior mesonephric, units.

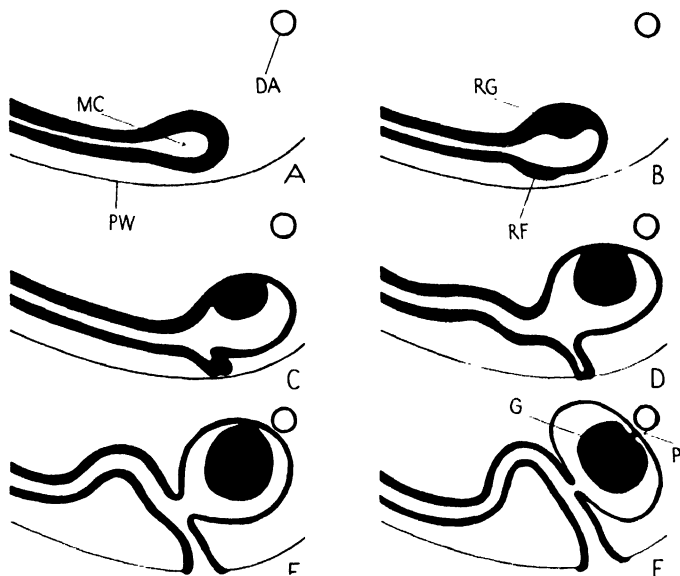
3. Posterior Mesonephric Units.—The subsequent history of the posterior mesonephric units, at any rate as regards their general shape, might very well be summed up in the one word 'swelling'. There are no new coils introduced into the simple tubule system of Henle's loop, which itself increases in size until the whole unit is spherical. A reconstruction of such a unit may be seen on figs. 11 and 12, Pl. 23, and in section on figs. 3, 5, and 6, Pl. 22, these last being as nearly as possible through similar planes to figs. 1 and 2 on the same plate. The only portion which has become further differentiated from the early unit type is the dorsal wall of the malpighian capsule, while the rudiments of a still-unopened peritoneal funnel are beginning to be developed.

The course of the development of the funnel is shown in full in Text-figs. 3 A to 3 F. In the case of the posterior mesonephric units now under discussion, the funnel never develops a clear connexion with the coelom, but ceases development at about the stage represented at fig. 3 c and on Pl. 22 at *rp*f, fig. 6.

The thickening of the upper wall of the malpighian capsule is of particular interest, since it is from this thickening (*rg*)—and not from an ingrowth of blood-vessels—that the actual malpighian glomerulus is derived. Both this process, as well as the full development of the peritoneal funnel, will be dealt with in detail when we come to discuss the development of the anterior mesonephric, or sexual, units.

The two sections already quoted show about the highest stage of development which is ever reached by these posterior mesonephric units. There is never any open connexion either from

the coelom or to the archinephric duct. At all stages up to and even after metamorphosis one may find examples of these deeply staining units embedded in the dorso-median angle of the definitive kidney. The actual solid attachment to the archinephric duct is severed shortly before metamorphosis when the



TEXT-FIG. 3.

Series of diagrams representing the development of the malpighian glomerulus and peritoneal funnel. *DA*, dorsal aorta; *G*, glomerulus; *MC*, malpighian capsule; *P*, position where the arterial connexion to the aorta will be formed; *PW*, wall of peritoneum; *RF*, funnel rudiment; *RG*, glomerulus rudiment.

great increase in the number of definitive tubules is rapidly driving the duct away from its previously median dorsal position.

To sum up, then, we may say that the mesonephros in the posterior region, is composed of a series of degenerate remnants which never achieve functional importance.

4. Sexual, or Anterior Mesonephric Units.—The sexual kidney of the adult consists, in the male, of some five

to seven units, more or less equally spaced out anterior to the definitive kidney. Each unit is clear and distinct, being joined from its median angle to the gonad by a *vas efferens* which opens into the malpighian capsule. A long, thin tubule connects each unit to the archinephric duct. Immediately posterior to the sexual units there is a short, anterior prolongation of the definitive kidney mass which is joined to both the archinephric duct and to the gonad; it would, perhaps, be more accurate to say that the last few sexual units have become mixed with the anterior end of the definitive kidney. The sexual region in the female is not nearly so sharply defined, the individual units not being distinct to the naked eye but forming a uniform anterior prolongation of the definitive kidney.

The development of the sexual units proceeds along the lines described in the section 'General Mesonephric Development' up to about the stage figured in Text-fig. 2 c, or in figs. 1 and 2, Pl. 22. This point is reached about a week after hatching before there is the least trace of development among the posterior nephroblast vesicles.

The differentiation of the sexual unit commences, about the time that Henle's loop becomes apparent, with the formation of a solid outgrowth from the junction of the stalk with the archinephric duct; this lateral branch, shown at *lb* in the two plate figures to which reference is made above, grows very rapidly, especially at the tip, so that it achieves a form with a thick, round base (*lb*, fig. 1, Pl. 22) and a thin, spatulate tip (*lb*, fig. 2, Pl. 22). This tip then thickens up so that the whole outgrowth assumes a clavate form. A lumen is then developed and the whole unit assumes the form shown in the reconstruction at fig. 9, Pl. 23. The presence of a basal lateral outgrowth is typical of, and confined to, units in the sexual region.

Let us now turn to the development of the malpighian capsule and its attendant peritoneal funnel. The thin walls of the developing tip of the club-shaped stage now commence to thicken rapidly at two points. The first of these points, which might be designated as the 'roof' of the developing capsule, is shown at *rg* in figs. 1 and 2 on Pl. 22. This thickening, which is the rudiment of the developing malpighian glomerulus, is

produced by the transformation of the squamous epithelium into columnar epithelium at this point. The second point of thickening is that area of the 'floor' of the developing capsule which immediately surrounds the attachment of stem to capsule. This thickening, which is the rudiment of the peritoneal funnel, is produced by a rapid proliferation of cells from the stem; in the case of the posterior mesonephric unit shown in fig. 5, Pl. 22 (which in this respect resembles the anterior), this proliferation of cells is clearly shown.

We therefore arrive at a condition diagrammatically shown in Text-fig. 3 B, where the same reference letters as those upon the plate are employed.

The cells forming the rudiments of the glomerulus and peritoneal funnels now commence to increase rapidly in number. The former create a definite mass of tissue which projects into the cavity of the capsule from the dorsal region, while the funnel rudiment grows downwards and presses against the peritoneal wall, first as a solid outgrowth but later as a blindly ending tubule. This gives the condition shown in Text-fig. 3 c.

While these changes are occurring in the head of the developing sexual unit, the increase in size, and coiling, of the tubule tend to force the developing capsule upward and inward towards the dorsal aorta. This movement further produces a rotation of the long axis of the capsule, since the tubule end of this is now attached, by means of the funnel rudiment, to the wall of the peritoneum. The glomerulus rudiment is therefore brought round from the dorsal to the dorso-median position opposite the aorta. These changes in the relative positions of the developing glomerulus are shown in Text-figs. 3 c to 3 f. A blood-vascular connexion is later formed in the position indicated by the arrow (*P*) in Text-fig. 3 f.

The development of the peritoneal funnel is more easily explained with the aid of diagram than in words. A comparison of Text-figs. 3 c to 3 f shows that, as the capsule rotates, the funnel rudiment is drawn out as a prolongation of that portion of the tubule from which it was originally proliferated; the thin-walled capsule remains as a lateral attachment to this neck. We are, therefore, left with a unit consisting essentially of a

coiled tubule connecting the coelom with the archinephric duct; at the duct end of this tubule a lateral branch grows out, while the funnel end is provided with a laterally attached malpighian capsule. This represents a typical sexual unit at an early stage; a slightly later stage, in which a few further coils are present in the main tubule, is seen in section at fig. 31, on Pl. 26. It is clearly demonstrated in this section that the malpighian capsule (*mc*), whose glomerulus (*mg*) has as yet no blood-vascular connexion, lies upon the side of the peritoneal funnel. The opening (*opf*) of this latter from the coelom obviously allows not only a clear passage through the tubule but also into the malpighian capsule. The lateral branch (*lb*) is, in this unit, just developing a lumen.

The further history of the sexual units is remarkable, since it consists of an over-elaboration of the lateral branch. Not only does this increase in length, but itself develops further lateral branches. The reconstruction shown in figs. 7 and 8 on Pl. 23 is taken from a larva about half-way to metamorphosis. There is a clear and distinct peritoneal funnel (*pf*), to the side of which is attached the malpighian capsule (*mc*). A short, darkly staining tubule (*n*) connects the funnel to the archinephric duct; the connexion is better seen in fig. 8 which is reconstructed from the dorsal angle. The main lateral branch (*lb*), growing out from the base of *n*, itself bears two subsidiary branches, labelled *2lb* and *3lb*. The origin of the former is best seen in fig. 8 and of the latter in fig. 9.

The reconstruction shown in fig. 10, on the same plate, is of particular interest since, in this case, there is no basal lateral branch. It seems more than probable, however, that the small outgrowth (*alb*) is an abortive attempt to form this branch. We find, moreover, that in this case the main branch (*mb*) itself bears a secondary lateral branch (*2lb*) about half-way along its length. The peculiar interest of this example will be discussed later in this paper.

Fig. 18 on Pl. 24 is taken through the reconstruction shown in figs. 7 and 8 on Pl. 23, just anterior to the attachment of the neck (*n*) to the archinephric duct (*ad*). The basal portion of the lateral branch (*lb*) is also visible in this section. Fig. 17, on the

same plate, is through the unit reconstructed in fig. 10, Pl. 23. In this may clearly be seen a downward prolongation of the neck (*n*) which leads to the peritoneal funnel a few sections later. The extreme anterior end of the malpighian capsule (whose cavity is indicated by the letters *cmc*) has just been cut as well as the margin of the now almost-completely, differentiated, malpighian glomerulus (*mg*).

To sum up, then, the development of a sexual unit, we may say that it rises extremely rapidly to a functional condition.

The future development consists of the production of blindly ending outgrowths whose significance is rather obscure. It seems not improbable that they represent the degenerate remnants of the secondary and tertiary units whose derivation from the definitive units will be shown later. It is difficult to account for their absence from the early posterior units unless one may postulate that the near presence of the gonadic strands stimulates the development of the anterior units.

DEVELOPMENT OF THE DEFINITIVE KIDNEY.

1. Origin.—The origin of the definitive units is identical with that of the mesonephric units, which, therefore, differ only in their rate of development and in their ultimate fate. It is perhaps difficult to see why the definitive units should be treated as distinct from the mesonephric units; the question hinges upon what may be meant by the word distinct. There is not, so far as the present writer can see, the slightest morphological difference between the nephroblast vesicles in the different regions of the body. Yet in the posterior it is unquestionable that certain of these vesicles develop into units which never arrive at a functional condition, whilst the remainder follow a course of development which differs widely from that of the anterior units.

The definitive kidney must be regarded therefore as a specialized structure which is distinct from the mesonephros only in so far that it is differentiated from this latter from the first moment that any trace of either becomes apparent. The most correct interpretation would probably be to regard all the excretory units as homologous in origin but becoming later

separated into series of units, each series being distinguished by certain embryological and morphological peculiarities which must themselves be obviously the product of functional adaptations.

2. **Primary Units.**—The definitive kidney, then, originates as a set of nephroblast vesicles derived from a solid rod of blastema. These become oval, develop an attachment to the archinephric duct and commence to coil, more or less, in the manner described for the mesonephric units on p. 434.

There are, however, certain differences which mark off the definitive unit from the neighbouring mesonephric units. In the first place there is the question of rate of development. Figs. 3 and 4 on Pl. 22 show sections through the similar planes of two units from the same larva. Fig. 3 (as also figs. 5 and 6) is through a posterior mesonephric unit at the highest point of its development. Fig. 4 is through a definitive unit which has already the beginnings of a derived unit showing. Yet the definitive unit in fig. 4 started development considerably later than the mesonephric unit in fig. 3. There is, then, a very definite, differential growth-rate. Though this becomes apparent, as we have just seen, from an examination of a single section, it is even more clearly displayed by examination of a series. Figs. 3, 5, and 6 on Pl. 22 are through the same unit. There might be some justification for confusing fig. 3 with fig. 4; but there could be no justification for confusing fig. 6 with fig. 16 on Pl. 24; yet both figs. 6 and 16 are through corresponding planes, the former of a mesonephric unit and the latter of the same definitive unit as that shown in fig. 4.

The development of these definitive units is very difficult to describe owing to the lack of a proper terminology for the different portions. In the description which follows, use is made of the terms usually applied to amniote metanephric tubules (Henle's loop, junctional tubule, &c.), but this is solely a matter of descriptive convenience. It is not in any way intended to indicate either a morphological homology or a similarity in function. The following definitions should render this quite clear.

1. 'Neck', a narrow, strongly ciliated tubule opening at one

end in a peritoneal funnel. The malpighian capsule is attached laterally to this tubule.

2. 'Junctional tubule.' A fairly thick tubule which forms the actual attachment to the archinephric duct.

3. 'Convolut ed tubule.' The entire length of tubule between 1 and 2 above. The convoluted tubule is divided into:

- (a) 'Proximal convoluted tubule.' That portion into which the neck opens.
- (b) 'Distal convoluted tubule.' That portion which immediately follows after the junction tubule.
- (c) 'Henle's loop.' That portion of the convoluted tubule which lies between the proximal and the distal. It is usually in the form of a fairly well-defined loop lying parallel to the archinephric duct.

There is, unfortunately, no apparent histological differentiation between the different parts, with the single exception of the ciliated neck; the other portions can neither be distinguished from each other nor, until shortly before metamorphosis, from the archinephric duct.

A fairly well-developed definitive tubule is shown in figs. 13 and 14 on Pl. 23. The short, thick, junctional tubule (*jt*) is attached to the dorsal surface of the archinephric duct (*ad*); this is, perhaps, better seen in section at fig. 4, Pl. 22. After making a sweeping curve in a posterior direction, the junctional tubule turns sharply back along itself to become the distal tubule (*dt*). A further reversal in direction gives Henle's loop (*hl*), which runs forward again into the proximal tubule (*prt*), which is itself joined by the neck (*n*); the actual junction is obscured in fig. 13 by the archinephric duct and in fig. 14 by the junctional tubule. It will be seen that the oval malpighian capsule (*mc*) is attached to the side of the neck.

The further development of the definitive units is bound up with the production of derived units and will be dealt with under this heading.

A word might be said at this point on the subject of the blood-supply. Text-fig. 4 is a transverse section through a developing definitive unit. The cavity of the malpighian capsule (*CMC*) contains an almost completely developed glomerulus which is

forming across the area *P* (compare Text-fig. 3 F) an arterial attachment to the dorsal aorta (*DA*): it is to be presumed that a venous attachment will be formed with the interrenal vein



TEXT-FIG. 4.

Photograph illustrating the blood-supply of a developing definitive unit. *AD*, archinephric duct; *CMC*, cavity of malpighian capsule; *DA*, dorsal aorta; *IR*, interrenal vein; *P*, as last fig.; *PCT*, posterior cardinal vein.

(*IR*), which has arisen as described for *Salamandra* by Hochstetter. So much for the glomerular supply. It is not, however, sufficiently realized that the blood-supply to the tubules from the posterior cardinal vein (to the right and above

the archinephric duct *AD*) is not capillary but sinusoid, the greater part of the length of the tubules actually lying within the blood-vessel. It should also be noticed that there is only an exceedingly thin membrane separating the cavity of the malpighian capsule from the blood-vessel.

3. Derived Units.--Returning for the moment to the definitive unit shown in figs. 13 and 14 on Pl. 23, we see that an outgrowth is commencing from the anterior bend of Henle's loop. This outgrowth is the rudiment of a secondary unit. That it is, in reality, an outgrowth from this bend, is shown by reference to fig. 16 on Pl. 24, where the end of the bend connecting the distal tubule and Henle's loop is cut on the right-hand side of the section. The solid stalk which is the origin of the secondary (*OS*) is quite obviously an outgrowth from the bend. This section also serves as a justification of the reconstruction and is similarly lettered.

The secondary unit develops a capsule and glomerulus in a manner analogous to the primary unit, save that the glomerulus rudiment arises from that portion of the stalk which is seen attached to the base of the capsule in fig. 4 on Pl. 22, where the lettering is again identical with that of the reconstruction on Pl. 23. The rudiment of the secondary glomerulus (*2rg*) is shown projecting into the developing cavity of the malpighian capsule (*2cmc*). It will be realized that by this method of production the capsule is naturally brought into that angle of the kidney where it may most conveniently form an arterial connexion with the dorsal aorta.

The reconstruction shown in figs. 23 and 24 on Pl. 25 is of a well-advanced, definitive unit with not only a functional secondary but also a developing tertiary. The arrow represents the direction of flow of the excretory products.

The primary malpighian capsule (*mc* in centre line) is attached, at its posterior angle, to the neck (*n*) a short distance behind the open peritoneal funnel (*pf* at centre of plate). The large, proximal, convoluted tubule (*prt* in centre line) is joined by the neck, the actual junction lying immediately under the archinephric duct (*ad* in margins), and then runs forward to execute an abrupt spiral before passing into the posteriorly directed.

limb of Henle's loop (*hl* in centre line). This continues back to the posterior limit of the unit, comes forward again in an anteriorly directed loop (same lettering) and bends sharply back upon itself to give the distal convoluted tubule (*dt* in right margin), which soon passes into the junctional tubule (*jt* in both margins). The actual attachment to the archinephric duct is hidden by the other tubule in fig. 24 and by the duct itself in fig. 23; the position is indicated in this latter figure by the labelling line *OP* from left margin.

Figs. 19 and 21 on Pl. 24 deal with the primary tubules just described. Fig. 19 is through the abrupt spiral which separates Henle's loop from the proximal tubule and is approximately through the lettering line *OS* (from left margin). Fig. 21 is approximately through the labelling line *OP* (also from left margin) and shows the insertion of the junctional tubule (*jt*) on the archinephric duct (*ad*); the connexion of the cavity of the malpighian capsule (*cmc*) with the neck (*n*) is also clearly shown in this section. Both limbs of Henle's loop (*hl*) are also cut, whilst the difference in size between the distal (*dt*) and proximal (*prt*) tubules is also well brought out.

Now let us examine the condition of the functional secondary unit which is attached to the anterior end of the primary unit just described. The secondary peritoneal funnel (*2pf* in centre line) bends sharply over the secondary malpighian capsule (*2mc*), the actual connexion between the two being on the ventral surface and therefore seen in fig. 24; it is also shown in section in fig. 32 on Pl. 26. The short neck (*2n* in right margin) is inserted into one side of the end of the large, proximal tubule (*2prt* in right margin); the present writer is unable to offer any explanation for the overlap of the proximal tubule beyond the neck, a feature which is well marked in most primary and secondary units. The proximal tubule runs backwards for some distance before turning sharply forward into a double bend which probably represents Henle's loop (*2hl* in right margin). The distal tubule (*2dt* both margins) runs forward a short distance before passing into the posteriorly directed junctional tubule (*2jt* in centre line). This junctional tubule opens at the point *OS* (left margin) into the abrupt spiral bend which

separates the primary proximal tubule from the primary Henle's loop.

Fig. 20 on Pl. 24 is through the attachment of the neck (*2n*) to the proximal tubule (*2prt*) and shows what is meant by the phrase 'inserted into one side of the proximal tubule' used above in the description of the reconstruction. The bend which forms the connexion between the junctional tubule (*2jt*) and the distal tubule (*2dt*) is also shown. The unlabelled tubules shown in this section belong to that unit which lies immediately anterior to the one under discussion and which has, for the sake of clarity, been omitted from the reconstruction.

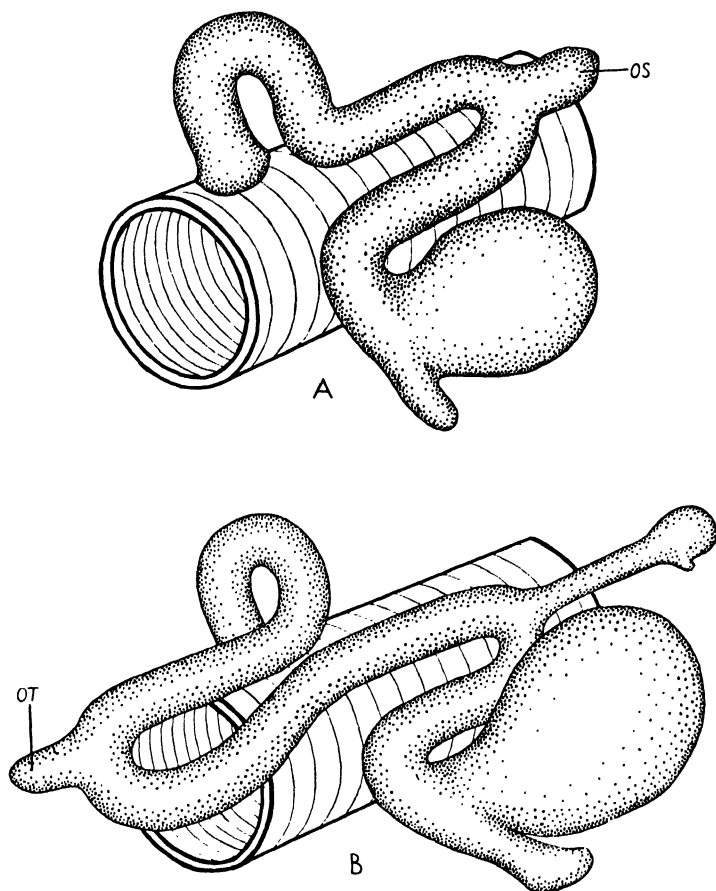
Turning to the posterior end of the reconstruction we find that there is here a developing tertiary unit. The tertiary malpighian capsule (*3mc* in centre line) lies upon the side of the tertiary neck (*3n*) which, after a series of convolutions analogous to those of the primary and secondary tubules, passes to the tertiary junctional tubule (*3jt* in centre line). The insertion of the tertiary tubule on the primary Henle's loop is indicated by the letters *OT'* (centre line).

The section shown as fig. 22 on Pl. 24 passes through the tertiary neck (*3n*) which is most clearly seen to be ciliated. The posterior coils of the primary Henle's loop (*hl*) and distal tubule (*dt*) are also cut by this section.

It should be noticed, though they have not been described in this order, that the secondary unit in figs. 13 and 14, the tertiary in figs. 23 and 24, and the secondary in the same figures form a developmental series of a derived unit.

Turning, for a moment, from the particular to the general, we may comment upon the fact that the coils of the developing unit follow so closely the plane of the archinephric duct; it must be remembered, however, that the whole developing kidney lies in relatively small area of retro-peritoneal tissue, which has never (as in *Rana*) been stretched to accommodate kidney blastema. Any increase in length of the tubules in *Triton* must, therefore, either be in an antero-posterior direction or must stretch a path for themselves as a protuberance into the coelom. Further, the method of coiling is very perfectly correlated with the method of production of the derived units,

since it results in the distribution of peritoneal funnels at equal distances in front and behind the primary. This is shown



TEXT-FIG. 5.

Diagrams illustrating the position of the derived definitive units.

OS, origin of secondary; *OT*, origin of tertiary.

diagrammatically in Text-figs. 5 A and 5 B, where *OS* is the origin of a secondary, and *OT* the origin of a tertiary, unit. The above description shows not only the necessity, but also some measure of justification for the adoption of a uniform

terminology. Not only may the primary and derived units be referred to a common plan, but we also find the derived units being given off from correlated parts of the primary. The present writer, however, would wish again to emphasize that in employing amniote, metanephric terminology, he is not suggesting a similarity of function. He realizes not only the necessity for a new series of tubule names but also that these names cannot justifiably be put forward until the whole range of amniote definitive kidneys has been thoroughly investigated.

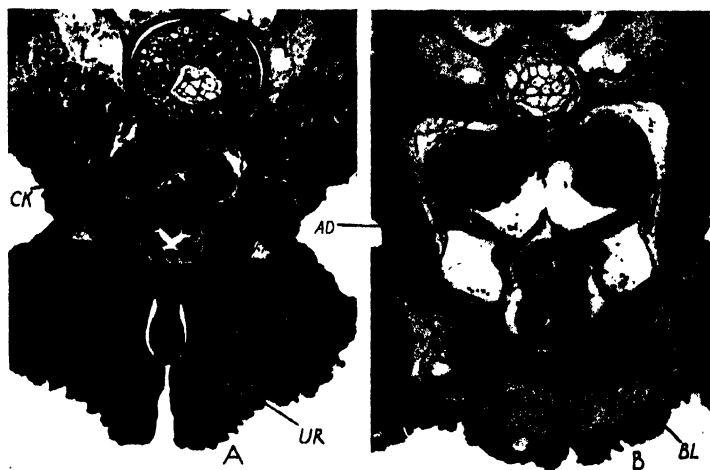
To sum up, then, the development of the definitive units, we may say that they are:

1. Derived from nephroblast vesicles laid down at the same time as those of the mesonephros.
2. Develop rapidly to a functional condition.
3. Give off further units by budding from the anterior and posterior bends of Henle's loop.

POST-METAMORPHIC DEVELOPMENT OF THE ARCHINEPHRIC DUCT AND URETERS.

1. Archinephric Duct.—At the time of approaching metamorphosis a series of histological changes take place in the archinephric duct. The cells of the wall shrink in size till little or no protoplasm remains visible around the nuclei. The duct, during this change, has been passing laterally across the kidney until it now projects as a ridge in the dorsal surface of the coelom; this is seen in fig. 25 on Pl. 26. There then appears to be a migration of connective tissue cells into this ridge with the result that the archinephric duct becomes embedded in a thick coating of connective tissue, as it appears in figs. 27 to 30 on Pl. 26. At the posterior end of the coelom the free, ventral edge of the ridge fuses with the peritoneal covering of the rectum for several millimetres, thus forming a mesentery down which the duct runs to open into the rectum. This is shown in Text-fig. 6 B, which is a transverse section of this region in a newt about a month after metamorphosis, the archinephric duct (*AD*) being about half-way down the mesentery; the section is a few millimetres in front of the opening of the bladder (*BL*) into the rectum.

Text-fig. 6 A is of particular interest in that it demonstrates in *Triton* the presence of a distinct posterior kidney, analogous to the 'caudal kidney' of many teleosts. The section is some three or four millimetres behind the opening of the archinephric ducts into the rectum and shows at *CK* that a coiled mass of solid kidney tubules projects into the most posterior region of the coelom. There can be no possible functional unit in this region since it could have no means of communicating with the



TEXT-FIG. 6.

Transverse sections of the posterior region of a young newt about a month after metamorphosis. *AD*, archinephric duct; *BL*, bladder; *CK*, caudal kidney; *UR*, urinary papilla.

exterior. In the female, the attachments of the primary tubules to the archinephric ducts remain throughout the life of the animal, being gradually drawn out as the duct recedes laterally from the kidney; it is these tubules which form the short transverse connexions throughout the length of the definitive kidney shown in Spengel's original figure (1876), so widely reproduced in modern text-books.

2. Male Ureters.—The post-metamorphic development of the male brings to light an extraordinary fact. The definitive units sever their connexion with the archinephric duct and

become re-attached to outgrowths of the latter; these outgrowths are the ureters. We will deal separately with the points raised by this statement.

First, the severance of the connexion with the archinephric duct. This process commences among the posterior units and progresses forwards, so that in examining a series of sections from the anterior to the posterior we should find successively:

- (i) Original connexion persisting.
- (ii) Original connexion degenerating.
- (iii) Original connexion severed.

These three stages are shown in figs. 25, 26, and 27 on Pl. 26. Fig. 25 shows the degenerating end of the junctional tubule (*djt*) which still, however, retains a distinct attachment to the archinephric duct. Fig. 26 shows the archinephric duct (*ad*) in free connexion with the junctional tubule (*jt*). In fig. 27 cells from the connective coating of this latter have migrated round at the point *ps* and completed the severance of the tubule. There seems, then, no reason to doubt that this severance actually takes place.

Now for the ureters. It is usually supposed that, in the male, the ureters are formed by the passing back along the archinephric duct of the original attachments of the junctional tubules. This would be, even on existing evidence, exceedingly improbable. Not only would the attachment have to creep down some forty or fifty millimetres within a period of less than a month, but it would have to do so along a duct thickly coated with connective tissue. Surely if this creeping were really to occur it would do so while the course was unimpeded.

A brief examination, moreover, of the condition in a newt about a month after metamorphosis at once shows that the usually accepted view must be wrong. For at this stage there are already five or six (the adult number) of ureters attached within the space of a millimetre to the posterior position of the archinephric duct. The most posterior of these are attached to the posterior units; the anterior ones are, as yet, attached only to the archinephric duct.

Let us turn to actual sections. Fig. 28 on Pl. 26 shows the attachment of a ureter (*ur 1*) to the archinephric duct (*ad*);

fig. 29 shows this same ureter passing towards the kidney tubules while fig. 30 shows it alongside the tubule with which, a few sections further forward, it fuses. In this last section is also shown another ureter (*ur* 2) which, when followed forward, is found to end blindly.

There is, therefore, no doubt whatever in the mind of the present writer that these ureters are in reality outgrowths from a short region of the posterior end of the archinephric duct. This is a point, unfortunately, the truth of which it is exceedingly difficult to demonstrate without recourse to actual sections. Photographs such as fig. 28, Pl. 26, can only very inadequately represent the 'budding' appearance which is so clearly shown in the original. The morphological condition described above must therefore remain the chief evidence for this mode of origin.

Now for the third point, the attachment of the severed end of the junctional tubule to the developing ureter. Let us, in the light of the facts given above, theoretically divide the post-metamorphic, definitive kidney into three regions—an anterior, a posterior, and a median. In the posterior region we find ureters joining definitive units to archinephric duct; in the middle region we find ureters attached by one end to the archinephric duct from which the definitive units are severing their connexion; in the anterior region we find definitive units attached to the archinephric duct but no ureters.

The present writer regards the correlation of these three facts as sufficient warrant for the statement that in Triton the ureters are outgrowths of the archinephric ducts which form secondary attachments to the existing definitive units, after these latter have severed their direct connexion with the archinephric duct.

Theoretical considerations arising from this interpretation of the facts will be considered later.

DISCUSSION.

1. On the Development of the Kidney in Triton.

We have seen, therefore, that in the development of Triton there appear two quite distinct sets of excretory units:

1. The early (mesonephric) units, which in the anterior region

develop rapidly to the functional condition in which they remain, but which in the posterior region never reach a functional condition and finally degenerate.

2. The later units, which develop into the definitive kidney of the adult. In the male, secondary connexions to outgrowths from the archinephric duct are developed.

It might be as well, at this point, to give some explanation of the use of the terms 'mesonephros' and 'definitive kidney'. The former is used to describe that series of units which arise throughout the length of the body posteriorly to the pronephros and which persists, in the anterior region of the adult, as the genital kidney. The term 'definitive kidney' is at first sight less easy of justification since there already exists the term 'opisthonephros'. This was put forward by Graham Kerr (1919) to cover 'many of the lower Vertebrates' in which 'there is no separation between mesonephros and metanephros, the two forming a continuous structure which acts as the functional kidney'. Now in Triton there is, in the course of development, a very distinct separation between two sets of units. There is an early set of units differentiated throughout the whole length of the nephric tract whose anterior members persist as the sexual kidney; and a later set, arising only in the region of the adult functional kidney to which they give rise. The early units, in fact, play no part either in the formation or in the composition of any adult structure other than the sexual kidney. Now such an origin and history is entirely consistent with what is known of the true mesonephros throughout the whole vertebrate series.

The definitive kidney, on the contrary, is derived from special units which, though they are of necessity essentially similar in origin to the true mesonephric units, are most certainly not 'continuous' with them.

If the present writer correctly interprets the definition of 'opisthonephros' quoted above it could only be applied to the kidney of Triton if the early posterior units reached functional maturity and played their part in the production of the adult functional kidney; such is not the case.

It seems, moreover, far better to retain the term 'meta-

nephros' in the sense in which it was used by Schreiner (1902) for the quite distinct, amniote, definitive kidney. Use is therefore made of the widely applicable term 'definitive kidney', since it would appear to be unsatisfactory either to make use of a morphological definition such as opisthonephros, which carries the implication of a course of development not found in *Triton*, or to erect a new morphological definition on the evidence of a single species.

It is not proposed to enter here into a detailed discussion of the work of previous writers. It should be sufficient to point out that the account given by Fürbringer (1877), though forming an excellent foundation for subsequent work, was based upon an examination of too few stages to permit him to realize the existence of both early (mesonephric) and later (definitive) units, while he was entirely misled as to the formation of the nephroblast vesicles. Clark (1881) pointed out that these latter were formed in *Amblystoma* by the breaking up of a solid rod of blastema. Hall, in 1901, gave a very full account of the development of the 'mesonephros' in the same form, but he again regarded the definitive kidney as a direct derivative of the earlier units. His account of the urodele, however, is far more dependable than his account of the anuran. None of the earlier workers reconstructed sufficiently advanced units to give any reliable account of the derived units.

There are two points in the account brought forward by the present writer which appear to warrant a detailed discussion. The first of these is the amazingly rapid development of the anterior mesonephric, or sexual, units and their subsequent behaviour. There can be no excretory necessity for these units within a week of the hatching of the larva, since the pronephros is, at this stage, actively functional. What other functional activity, then, could account for their production? Now, it will be remembered, that in *Rana* (Gray, 1930) the development of the early units is designed to provide a direct connexion between the coelomic fluid and the blood-supply at the earliest possible moment; it is improbable that there should be no functional explanation of this. It does not seem too much to suppose that there exists a similar functional necessity in

Triton which is fulfilled by bringing the coelomic fluid into osmotic connexion with the blood-supply. There is, of course, already an osmotic connexion through the glomeruli of the pronephroi and we must, therefore, seek some further reason why a connexion should also be established in the sexual region. Now it seems quite possible that the developing genital strand may, even at this early stage, be giving rise to an endocrine secretion. The quickest manner by which this secretion could reach the blood-stream would be by the anterior mesonephric glomeruli, since there is, as yet, no capillary irrigation of the developing gonad. It is quite possible, therefore, that the anterior mesonephros is rapidly developed to subserve the function of sexual kidney, a function which it continues to subserve throughout the whole life of the animal.

This explanation can also be correlated with the production of lateral branches which constitutes (p. 441) the further development of these units. If these units had been derived to subserve a purely excretory function, we might expect that their further development would be along lines destined to augment their excretory capacity; such is not the case. If, on the contrary, the production of a single, large, peritoneal funnel and its attendant glomerulus were sufficient to subserve the secondary function ascribed to them, the production of further blind tubules would be no more than the degenerate recapitulation of an ancestral condition. This is borne out by the lack of continuity of type shown in these units, exemplified by the reconstruction shown in fig. 10 on Pl. 23 and discussed on p. 441. It is far more probable that irregularities of structure would occur in a degenerating object than in one which is being developed to serve the needs of a special function.

The second point which appears worthy of detailed discussion is the secondary attachment of the definitive units to the male ureters, since this feature of Triton development appears at first sight difficult to correlate with previous accounts; but, so far as the present writer can find out, no previous worker has studied the development of the ureters. They have observed the attachment of the units in the older larvae and the attachment of the ureters to the hinder portion of the

archinephric duct in the adult. The correlation of these two facts with the statement that the attachments pass backwards along the archinephric ducts, appears in every case to be a theoretical assumption made without further investigation of intermediate stages.

It is, however, of great interest to find a not fundamentally dissimilar process taking place in *Hypogeophis*. Brauer (1902) states that the derived units of the definitive kidney of this form arise as buds from the nephrotome which sever their connexion with this latter and become attached to a secondary outgrowth from the archinephric duct. There must, of necessity, be an immense evolutionary gap between *Hypogeophis* and *Triton*, but it is none the less of interest to find the same fundamental principle—the budding of the archinephric duct—being shown in the development of the definitive kidney of both.

2. On the Comparison of this Development with that in *Rana*.

(a) Early, or True, Mesonephric Units.

In both forms an early set of units—the mesonephros—develop and subsequently degenerate. In *Triton*, and probably also in *Rana*, the anterior mesonephric units persist as the sexual kidney. In *Triton* blind diverticula occur as outgrowths from the sexual units. The chief argument against these tubules being excretory is the lack of any necessity for them to subserve such a function in a larva furnished with an actively functional pronephros; there appears to be nothing to prevent aglomerular units (or, by analogy, an aglomerular outgrowth from a unit) from functioning (Marshall and Grafflin, 1928). It seems, however, not improbable that the production of blind diverticula by a mesonephric unit may be a far more common phenomenon than is usually realized. They have already been observed in *Myxocephalus octodecimspinosus* (Nash, 1931) and in certain snakes (Régaud and Policard, 1908), so that it seems safe to suggest that they may be found in many other forms and that a discussion as to their function is better left until a further knowledge of their morphology has been obtained.

(b) Definitive Kidney.

The definitive, or adult excretory, kidney is not, in either *Rana* or *Triton*, a direct derivative of the early mesonephric units, but is developed independently from primary definitive units to which are later added secondary and tertiary units. In order to understand the correlation between the methods by which these secondary and tertiary units are added, it is necessary to remember that in *Rana* there is a large quantity of loose nephrogenetic tissue, known as the blastema, lying in the mediodorsal angle of the developing kidney; in *Triton* there is no loose blastema. In this latter form, therefore, the secondary and tertiary units are derived by the budding of the primary definitive unit which itself represents the whole of the available supply of blastema.

Now in *Rana* the primary definitive units never reach a functional condition but are repressed into the function of collecting trunks for the derived definitive units. This trunk, which is referred to in Part I as the 'straight tubule', develops (Text-fig. 6, Part I), from a nephroblast vesicle in a manner exactly analogous to the production of the primary definitive units in *Triton*. The derivative definitive units, which in this latter form are wholly budded off from the primary (Text-fig. 5, Part II), are formed in *Rana* partly from the available reserve of blastema (Text-fig. 7, Part I), only the functional tubules being budded off from the straight tubules. There is, therefore, no morphological objection to the statement that the definitive units of both forms are homologous, both in origin and in structure. Further, the variation in detail of the method of production can be correlated with the general development of each form. This was pointed out in Part I. 'An ancestral form, with a long larval life and consequently no need to produce an adult kidney in the quickest possible manner, has given rise first, to the Urodelan kidney with its fairly rapid method of production . . . and secondly, the kidney just described (*Rana*) in which the period occupied by the Urodeles in the formation of a nephrically functional, collecting trunk has been suppressed, and further, in which a method of producing a

number of malpighian units in quick succession has been evolved.'

(c) Function.

There remains the problem of the peritoneal funnels. In *Rana* these are arranged to carry the coelomic fluid directly into the blood-stream; this function appears so important that a later set of funnels, derived from a special funnel-forming tubule (Text-fig. 8, Part I), retains no developmental trace of ever having subserved any other function. It is difficult to see how this connexion between coelom and haemocoel can assist in excretion, even though the funnels remain actively functional in the adult. It is probable, indeed, that this retention of actively ciliated funnels may be correlated with the very dense nerve-net, which has recently been shown (Hirt, 1930) to exist on the ventral surface of the kidney in *Rana*.

As was pointed out in the conclusions appended to Part I of these investigations, there seems no reason why a purely excretory function should be attributed to the peritoneal funnels. If we are to accept the suggestion put forward that these funnels collect an endocrine secretion from the developing gonad, then the exceedingly rapid production of funnels by *Rana* is no more than the expression of the shortened larval existence shown by this form. In *Triton*, however, the coelomic fluid is in osmotic connexion with the blood-supply and it might be argued that the usual direction of osmotic flow is from the glomerulus to the capsule. Yet modern research tends more and more to show that the osmotic membrane of a kidney glomerulus is extremely selective and there is nothing to show that it may not be reversible. It seems certain, indeed, that there must be reversibility in the wall of Bowman's capsule, otherwise (Text-fig. 4, Part II) there would be a heavy osmotic leakage from the capsule into the posterior cardinal vein! In fact, it may well be asked how far the amphibian glomerulus is ever concerned in excretion. Bensley and Steen, in 1928, showed that many substances were excreted by the frog's kidney tubules after all possibility of glomerular circulation had been removed by ligaturing, and it has even been asserted (Richards and Barnwell,

1927) that an excised frog's kidney, washed free from blood, concentrated certain dyes from a solution in which it was immersed, directly into certain portions of the tubule. It may be objected that the present writer is basing assumptions as to function upon morphological data. The original assumption of Bowman (1842) as to kidney function was based upon nothing else, and while it is not intended for one moment to suggest that the amphibian glomerulus never excretes, there appear to be considerable grounds for suggesting that there may be, at any rate in the mesonephros, the secondary function of extracting something from the coelomic fluid. It is interesting to note that no aglomerular kidney (Marshall and Grafflin, 1928; Edwards, 1928; Marshall, 1929) is ever furnished with peritoneal funnels, and the present writer cannot agree with the assumption of Marshall and Smith (1930) that 'the "protovertebrate kidney" was originally aglomerular and the glomerulus evolved as an adaptation to fresh-water habitat'. It is further interesting to find the suggestion of Buchanan and Fraser (1919) that the anterior mesonephros of marsupials (in which the peritoneal funnels are vestigial) may function as an aglomerular kidney.

3. On the Comparison of these Developments with those in other Amphibia.

There is no need here to recapitulate the many accounts of the development of various Amphibia which have been brought forward. Such accounts are to be found summarized in most modern text-books (see especially Kerr, 1919; Brachet, 1921; Goodrich, 1930), and the more important have already been noticed either in the Introduction to Part I or in the present discussion.

There are, however, two points in the present writer's account which it would be interesting to bring into line with existing data for other forms. These points are the distinction between the early (mesonephric) and later (definitive) units, and the attachment of these latter to the archinephric duct.

The first point has never been brought out in any previous account, but one is inclined to think that Hall (1904) wanted to believe what his insufficient data did not disclose. He

comments very strongly on the fact that the (posterior) 'later units' are delayed in development and points out that there is a 'sudden (*sic*) transition, in the region of the sixteenth or seventeenth somite, from complex units to simple blastulae'. It is possible, of course, that the posterior mesonephric units are entirely suppressed in *Amblystoma* and that the delayed units—obviously corresponding to the primary definitive units of *Triton*—are the only existing ones. Again let us quote from Hall: 'The posterior portion of the excretory part is thus distinguished from the anterior portion by an anatomical character—the presence of dorsal units. Significance is given to this difference in anatomical character by a peculiarity in the development of this region of the mesonephros which, though not conspicuous, is to be considered of some importance from a phylogenetic standpoint. This peculiarity consists in a retardation in the appearance and development of the blastulae (*sic*).' It seems very possible, then, that if Hall had reconstructed later units from both the anterior and posterior regions, the present writer would not be the first to point out that the anatomical differences between the two are sufficient to justify the posterior set being separated off as definitive kidney units. The present account, of course, depends not only on these anatomical differences, but also on the demonstrable presence of both early and definitive units in the hind region and on the subsequent degeneration of the early set.

The second point on which it appears desirable to comment is the secondary attachment of the definitive units to the archinephric duct. Now it appears to the present writer that this may not be a newly evolved feature peculiar to the male *Triton* but a primitive feature which is reproduced in *Triton* only in the male. Both Brauer's account of the development of *Hypogeophis* (1902) and Semon's account of *Ichthyophis* (1892) show that in these amphibians the definitive units are attached to outgrowths from the archinephric duct; it has been shown by the present writer (as by all previous observers) that no such outgrowths arise in *Rana*. If, therefore, we accept that *Gymnophiona*, *Urodela*, and *Anura* represent an evolutionary series for *Amphibia*, and if we accept as respec-

tively typical of these groups the course of kidney development shown by *Hypogeophis* (Brauer), *Triton* (mihi), and *Rana* (mihi), we may then show an evolutionary series for the amphibian kidney:

A. *Gymnophiona*.

1. *Mesonephros*.—An early set of units arising at outgrowths from the nephrotome. The connexion with this latter is retained as the peritoneal funnel while the opposite end of the unit grows into the archinephric duct.

2. *Definitive Kidney*.—A later set of units which are budded off from the nephrotome. Two outgrowths arise from these vesicles, one of which gives rise to a peritoneal funnel while the other fuses with an outgrowth from the archinephric duct.

B. *Urodela*.

1. *Mesonephros*.—The nephrotomes are fused into a continuous rod of blastema. This gives rise to an early set of units which in the posterior region never reach maturity but in the anterior persist as the sexual kidney. There is a direct connexion to the archinephric duct.

2. *Definitive Kidney*.—A later set of units arise from a distinct set of vesicles. These primary definitive units serve as collecting trunks for the later definitive units to which they give rise by budding. In the male the archinephric duct retains its power of giving rise to outgrowths which are delayed till after a transitory connexion has been formed between the collecting trunks and the archinephric duct.

C. *Anura*.

1. *Mesonephros*.—The nephrotomes break down into a mass of loose blastema tissue, in which an early set of condensed vesicles give rise to an early set of units which subsequently degenerate. These units form a direct connexion to the archinephric duct.

2. *Definitive Kidney*.—A later set of suppressed units arise which serve as collecting trunks for the derived definitive units which are themselves derived partly from the collecting

trunk and partly from the blastema mass. There is no budding from the archinephric duct.

We find, then, that in all Amphibia:

1. An early set of units which, on the evidence of *Gymnophiona*, would appear to be serially homologous with the pronephros and to which the term 'mesonephric' may be justifiably applied.

2. A later set of posterior definitive units, each of which, in *Gymnophiona*, develops an individual connexion with an outgrowth from the archinephric duct. In *Urodela* some of the earliest of these later units develop a direct connexion with the archinephric duct, bud off the derived units and then, in the male, acquire connexions with outgrowths from the archinephric duct.

The fact that the derived definitive units in *Urodela* are budded from the primary definitive units is no more than an expression of the fact that these latter represent the only available source of intermediate mesoderm.

There would, therefore, appear to be a continual evolutionary effort to shorten the time required to produce a definitive kidney by the substitution of a modified unit (collecting trunk) for an outgrowth from the archinephric duct. The male urodele, however, reverts to the primitive type, presumably in order to separate the sperm from the products of excretion, a process apparently not necessary to *Rana*.

To sum up, then, the evolution of the method of attachment of the amphibian definitive kidney, we may point to three existing stages:

1. No collecting trunk but direct connexions to outgrowths from archinephric duct (*Gymnophiona*).

2. Direct connexion to archinephric duct through nephrically functional collecting trunk reverting, in male only, to a connexion with outgrowths from archinephric duct (*Urodela*).

3. Direct connexion to archinephric duct through highly modified collecting trunk (*Anura*).

The origin of the definitive kidney appears in all cases to be the same—that portion of the intermediate mesoderm which remains after the production of the early, true mesonephric units.

In conclusion, the author would like to express his gratitude for the sympathetic encouragement which was afforded him by Professor E. W. MacBride, F.R.S., in whose Research Laboratory at the Imperial College of Science this work was carried out, and to Professor E. S. Goodrich, F.R.S., whose constructive criticisms have materially improved the theoretical portions of this paper. The author is further glad of this opportunity to thank Miss D. E. Sladden, to whom he is indebted for much of his material, and Messrs. H. R. Hewer, M.Sc., and T. L. Green, B.Sc., for their very practical assistance.

SUMMARY AND CONCLUSIONS.

1. A description is given of a modified reconstructional technique used in the study of advanced units.

2. It is shown that, in Triton, the definitive kidney is not a derivative of the original mesonephric units, but is of distinct origin.

3. (a) The mesonephros arises from a set of nephroblast vesicles, themselves derived from a solid rod of blastema, extending from the anterior limit of the genital strand to the posterior limit of the coelom.

(b) These nephroblast vesicles develop as shown in Text-fig. 2.

(c) The mesonephric units in the posterior region never reach a functional condition and subsequently degenerate.

(d) i. In the region of the developing gonad the mesonephric units develop rapidly to a functional condition.

ii. A basal lateral outgrowth appears near the junction of the developing unit with the archinephric duct.

iii. This outgrowth develops into a blindly ending tubule, which itself gives off other tubules.

4. (a) The primary definitive-kidney units arise from nephroblast vesicles similar to those of the mesonephric units, but present only in the posterior region.

(b) These develop similarly to the mesonephric vesicles (Text-fig. 2) but no basal lateral outgrowths appear.

- (c) The detail of the production of the malpighian capsule and peritoneal funnel is given (Text-fig. 3) and the blood-supply discussed (Text-fig. 4).
- 5. The derived definitive units arise as buds from the anterior and posterior bends of Henle's loop of the primary definitive units (Text-fig. 5).
- 6. (a) The archinephric duct passes ventro-laterally away from the main mass of the kidney and now forms a ridge in the dorsal peritoneum.
(b) Connective tissue cells migrate into this ridge and form a thick covering to the archinephric duct.
- 7. (a) In the male the definitive units sever their connexion with the archinephric duct.
(b) Outgrowths appear from a small posterior area of the archinephric ducts; these outgrowths are the ureters.
(c) These ureters grow forward parallel to the archinephric ducts and form connexions with the definitive units.
- 8. (a) It is explained why the term definitive kidney has been employed in place of opisthonephros.
(b) It is suggested that the extraordinarily rapid growth, and curious production of blind tubules by the sexual units, may be correlated with the possibility of their collecting an endocrine secretion from the developing gonad, which is at this stage without blood vascular irrigation.
(c) It is shown that a precedent for the secondary attachment of the definitive units to outgrowths from the archinephric duct is to be found in *Hypogeophis* (Brauer, 1902).
- 9. It is shown that the course of development in both *Rana* and *Triton* is fundamentally similar in that both forms—
(a) Develop two sets of units—the early (true mesonephric) units and the later (definitive kidney) units.
(b) The early units play no part in the production of the functional units of the adult.
- 10. The definitive units of both are also similar if the 'straight tubules' of *Rana* (Gray, 1930) be homologized with the 'primary definitive units' of *Triton*. The differences in the

development of the derived definitive units are due to differences in the character of the remaining intermediate mesoderm.

11. It is suggested that the mesonephric glomeruli and peritoneal funnels subserve the additional function of passing something from the coelomic fluid to the blood-stream.

12. If the attachment of the derived definitive units to outgrowths from the archinephric duct found in *Gymnophiona* is a primitive feature, then an evolutionary series for the amphibian kidney may be shown thus:

- (a) All definitive units attached to outgrowths from the archinephric duct (*Gymnophiona*).
- (b) Outgrowths from the archinephric duct in male only (*Urodela*).
- (c) No outgrowths from the archinephric duct (*Anura*).

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EXPLANATION OF PLATES 22 TO 26.

LIST OF COMMON ABBREVIATIONS.

a, point of attachment of developing unit to *ad*; *ad*, archinephric duct; *alb*, abortive *lb*; *bl*, blastema; *cmc*, cavity of *mc*; *dg*, developing gonad; *djt*, degenerating *jt*; *dt*, distal tubule; *hl*, Henle's loop; *jt*, junctional tubule; *lb*, lateral branch; *mb*, main branch; *mc*, malpighian capsule; *n*, neck; *ofm*, opening of *pf* to *mc*; *opf*, opening of *pf* to *n*; *OP*, origin of primary; *OS*, origin of secondary; *OT*, origin of tertiary; *pf*, peritoneal funnel; *ps*, point of detachment of *jt* from *ad*; *prt*, proximal tubule; *rg*, rudiment of *mg*; *rpj*, rudiment of *pf*; *ur*, ureter.

The figures '2' or '3', prefixed to any of the above abbreviations, indicate respectively secondary and tertiary derived structures.

PLATE 22.

Figs. 1 and 2.—Transverse sections of a rudimentary sexual unit from an embryo about to hatch.

Figs. 3, 5, and 6.—Transverse sections of a posterior mesonephric unit from a larva about a month after hatching.

Fig. 4.—Transverse section of a definitive unit from the same larva as figs. 3, 5, and 6 above.

PLATE 23.

Figs. 7 and 8.—Ventral (7) and dorsal (8) reconstructions of a sexual unit in a larva about a week after hatching.

Fig. 9.—Dorsal reconstruction of a developing sexual unit.

Fig. 10.—Dorsal reconstruction of a slightly abnormal sexual unit from a larva about a week after hatching.

Figs. 11 and 12.—Ventral (11) and dorsal (12) reconstructions of the posterior mesonephric unit shown in figs. 3, 5, and 6 above.

Figs. 13 and 14.—Ventral (13) and dorsal (14) graphic reconstructions of a developing definitive unit from a larva about a month after hatching.

PLATE 24.

Fig. 15.—Transverse section of 'right shoulder' region in a newt embryo about a week before hatching.

Fig. 16.—Transverse section of the developing definitive unit shown in figs. 4, 13, and 14.

Fig. 17.—Transverse section of the sexual unit shown in fig. 10.

Fig. 18.—Transverse section of the sexual unit shown in fig. 9.

Figs. 19 to 22.—Transverse sections across the developing definitive unit reconstructed in figs. 23 and 24. The approximate planes are indicated in the text.

PLATE 25.

Figs. 23 and 24.—Ventral (23) and dorsal (24) graphic reconstructions of a well-developed definitive unit.

PLATE 26.

Fig. 25.—Transverse section showing degenerating functional tubule from a larva about a week before metamorphosis.

Figs. 26 and 27.—Transverse sections to show relations of junctional tubule to archinephric duct in a young Triton about a month after metamorphosis.

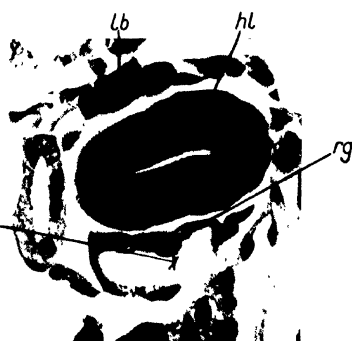
Figs. 28–30.—Transverse sections to show ureters in same larva as figs. 27 and 28 above.

Fig. 31.—Transverse section to show relations between the various parts of a newly functional sexual unit in a larva about a week after hatching.

Fig. 32.—Transverse section to show relations of the various parts in a functional secondary unit.



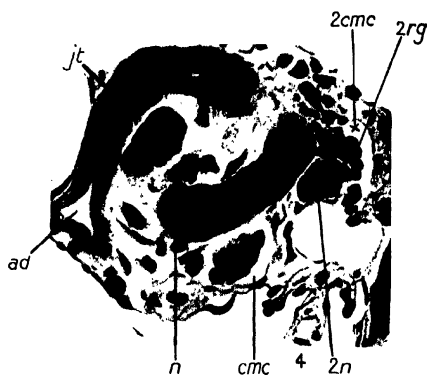
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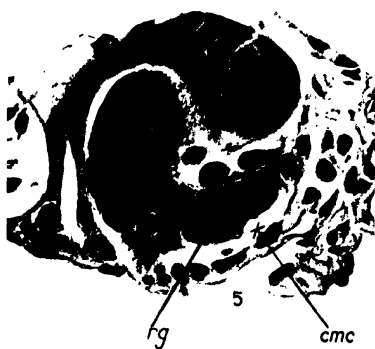
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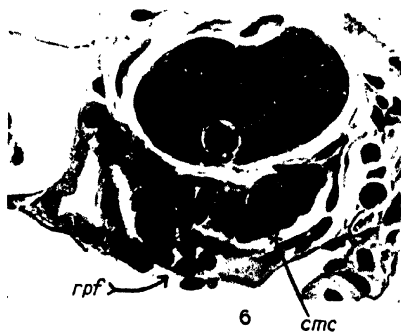
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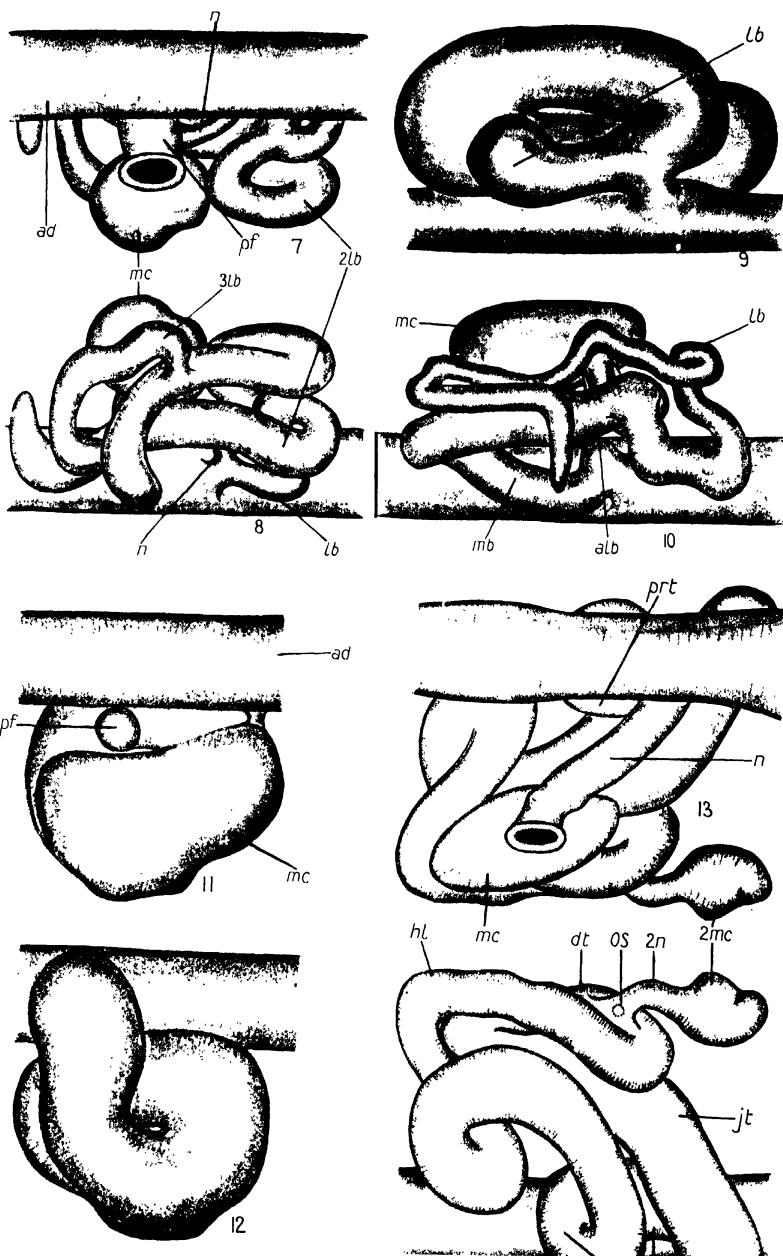
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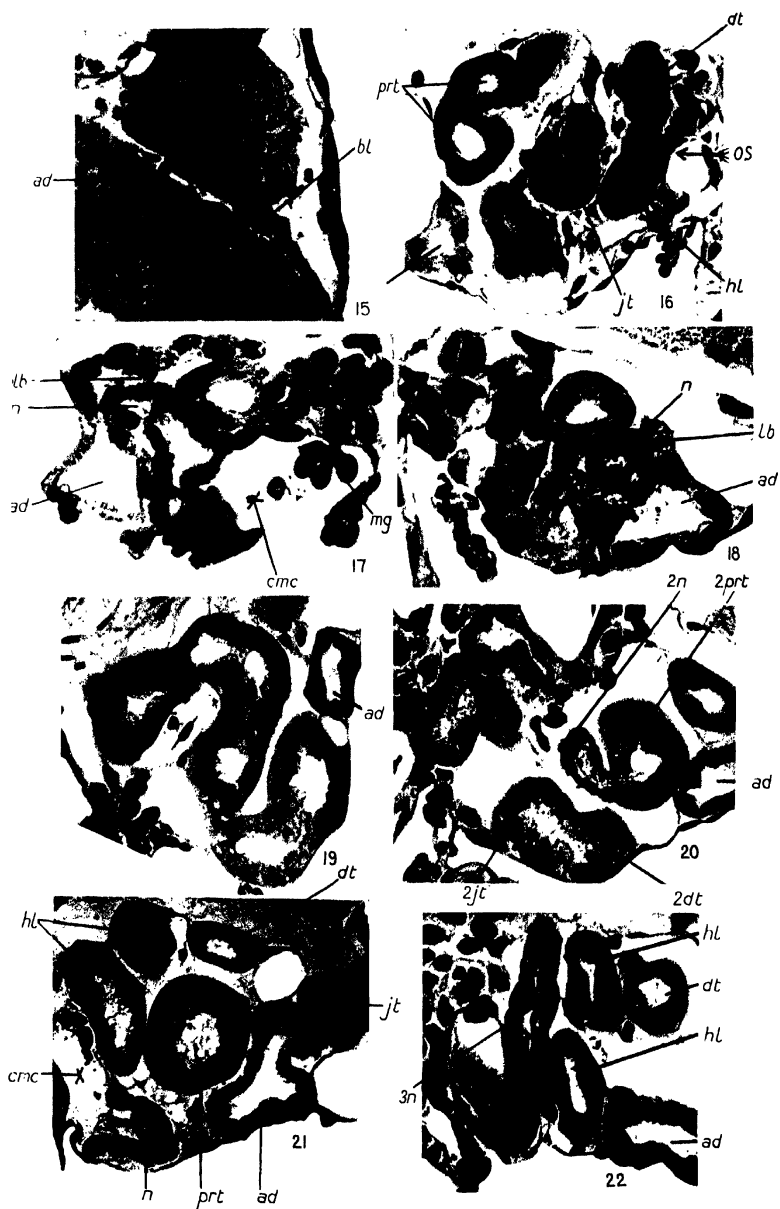


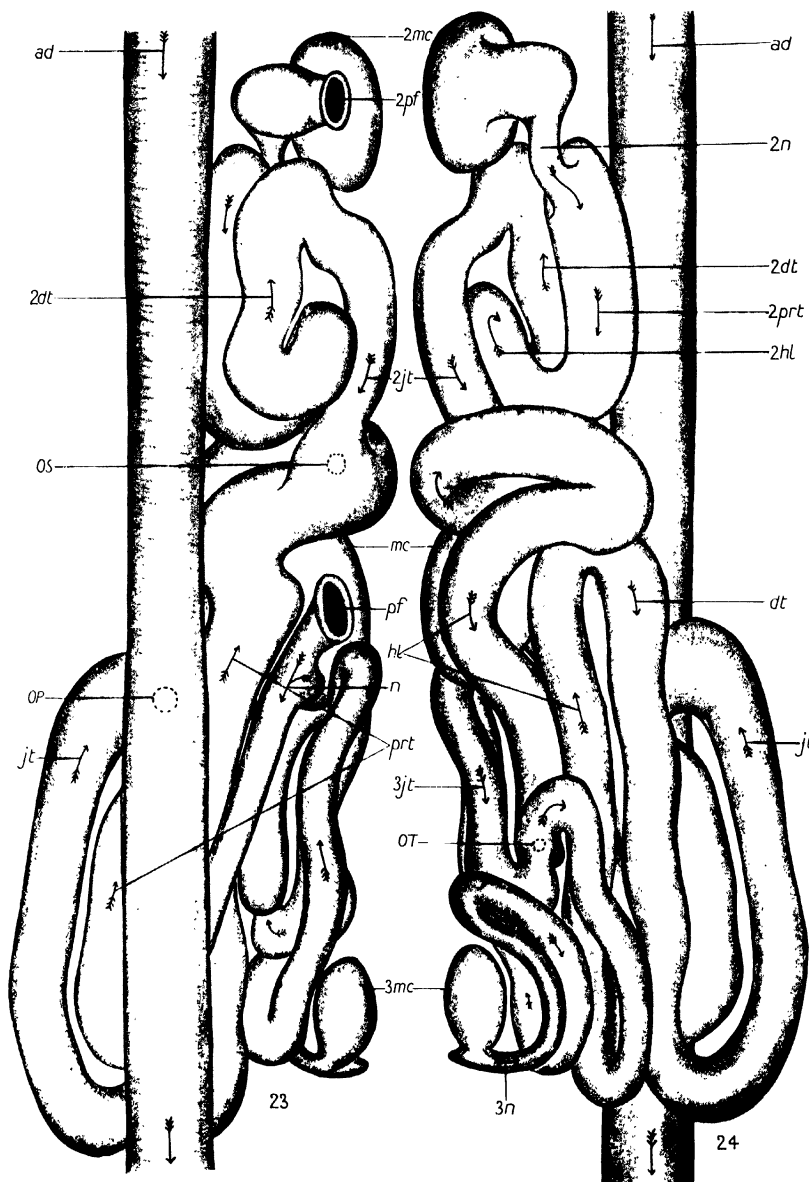
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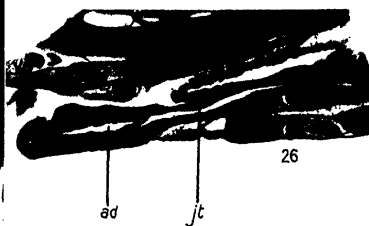




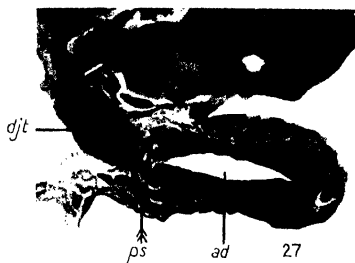




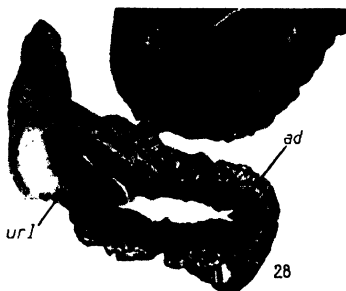
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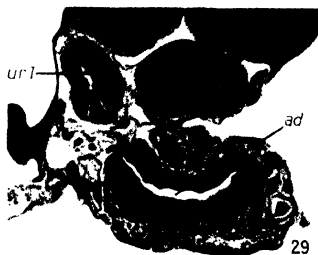
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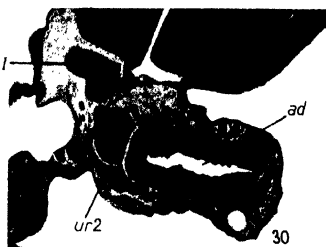
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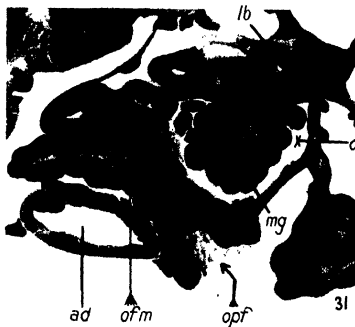
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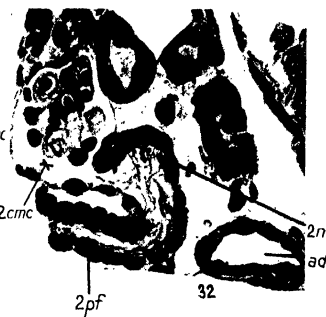
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Notes on the Structure and Development of the Reproductive Organs in *Philaenus spumarius* L.

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With Plates 27 and 28.

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I. INTRODUCTION.

THE structure and development of the reproductive system in the Hemiptera-Homoptera have already been investigated by Christophers and Cragg (3), Kershaw and Muir (6), Singh Pruthi (12 and 13), and George (4). As these authors differ considerably in their conclusions, the following work on *Philaenus* was undertaken in an attempt to elucidate some of the doubtful points.

Material for the study, collected from *Rumex* spp. and *Carduus* spp., was preserved and sectioned in the usual manner. The work was carried out in the Department of Zoology, University College of Wales, Aberystwyth, under the supervision of Professor R. D. Laurie, M.A., to whom the writer desires to express her thanks for much valuable advice and criticism.

II. THE PROBLEMS PRESENTED.

The following are the chief problems presented in the study of the reproductive system:

A. The Genitalia.

- (i) The nature of the genital appendages, i.e. whether paired or unpaired in origin.
- (ii) The location of the genital appendages.
- (iii) The homologies of the appendages, i.e. whether derived from primary segmental appendages, and, if so, from which segments of these latter.

B. The Efferent System.

- (i) The location of the gonopore.
- (ii) The extent to which the primary efferent passages (i.e. the embryonic genital strands) are retained in the adult.
- (iii) The secondary development of ducts of an ectodermal nature, supplementing or replacing the original efferent passages.
- (iv) The nature of these ducts, i.e. whether paired or unpaired.
- (v) The point of origin of these ducts.
- (vi) The origin of the accessory genital organs, i.e. accessory glands, vesiculæ seminales, bursa copulatrix, and spermatheca.

III. THE MALE.

A. Adult Structure.

(i) The Genitalia.—The genitalia are situated on the ninth segment and consist of the median unpaired aedeagus which bears the gonopore at its apex, paired parameres, and sub-genital plates.

(ii) The Efferent System.—The efferent system comprises a pair of testes which are in communication with a pair of vasa deferentia, the ejaculatory duct, and the accessory glands.

The vasa deferentia are slender ducts of uniform calibre thrown posteriorly into a series of loops before receiving the openings of a pair of accessory glands. The ducts formed by the union of the vasa deferentia and accessory glands are short and straight, uniting in the ninth segment to form the median

ejaculatory duct. This latter traverses the aedeagus to communicate externally by means of the gonopore.

B. Development.

(i) The Genitalia.—In a young nymph, prior to the development of the pleural folds which are so characteristic a feature of the adults of *Philaenus spumarius*, the gonopore is bordered by a pair of outgrowths, the ectodermal cells of which are greatly enlarged (fig. 1, Pl. 27). These are the rudiments of the primary genital appendages. At a rather later stage of development, the ninth segment may be seen to bear two pairs of appendages, the ventral and anterior pair representing the rudiments of the sub-genital plates, the dorsal and posterior being the primary genital appendages (fig. 24, Pl. 28). The latter subsequently become divided to form two pairs of appendages, the inner and dorsal pair being the rudiments of the aedeagus, the outer and ventral, of the parameres. Fig. 9, Pl. 27, shows the aedeagus and parameres incompletely separated from each other and from the subgenital plates at their bases. Fig. 10, Pl. 27, shows the complete division of the primary genital appendages into paired rudiments of aedeagus and parameres. At a still later stage of development the rudiments of the aedeagus become fused to form a median tubular organ bearing the gonopore at its apex (fig. 15, Pl. 27). Basally, the aedeagus and parameres are still incompletely separated (fig. 14, Pl. 27).

(ii) The Efferent System.—The ejaculatory duct arises in the early nymphal instar, as an invagination of the ectodermal layer posterior to the ninth sternite. In this region the ectodermal cells are very greatly swollen (fig. 1, Pl. 27) and, as previously stated, form the rudiments of the primary genital appendages. The ejaculatory duct is very short and straight and immediately divides to form two lateral ducts (fig. 2, Pl. 27). A little later the ends of the lateral ducts become constricted in a horizontal plane to give rise dorsally to the lateral ejaculatory ducts, and ventrally to the accessory glands (fig. 3, Pl. 27). The accessory glands pursue a straight course anteriorly: the lateral ejaculatory ducts run transversely before making a forward bend to run parallel to the accessory glands (fig. 25, Pl. 28).

The essential elements of the efferent system being now formed, maturation consists of the elongation and elaboration of these parts.

The accessory glands increase greatly in length without undergoing any convolution.

Four growth centres are present in the ejaculatory duct, situated, one in the posterior unpaired region, another at the point of division of the median duct, and one at the base of each of the lateral ejaculatory ducts (i.e. anterior to the point of origin of the accessory glands) (fig. 25, Pl. 28).

As a result, the point of division of the median duct is carried much farther anteriorly, while the lateral ejaculatory ducts are thrown into two or more loops at their bases (figs. 11 and 12, Pl. 27; fig. 26, Pl. 28).

Each testis is composed of six testicular follicles situated in the anterior region of the abdomen. The follicles lead into vasa efferentia, which in their turn unite to form vasa deferentia.

Owing to a lack of material of a suitable age, the extent of the mesodermal vasa deferentia and the point of their union with the lateral ejaculatory ducts could not be determined.

C. Conclusions.

(i) The Genitalia.—(a) The genitalia, i.e. aedeagus, parameres, and subgenital plates, are paired in origin.

The development of the subgenital plates from a pair of primary appendages is easily followed. The aedeagus and parameres are derived from a pair of primary appendages which later become subdivided to form two secondary pairs, the inner of which fuse to form the median tubular aedeagus, the outer remaining distinct as the parameres.

A similar mode of development is described by Singh Pruthi (13).

George (4) states that the aedeagus arises as a pair of lobes in the young which, during later stages, fuse to form the copulatory organ. Quoting from his paper:

‘The parameres of Homoptera are not structures of any morphological significance as assumed by some authors, but are outgrowths of the aedeagus.’

The conclusions of George, therefore, do not differ fundamentally from those of Singh Pruthi and myself, since in both cases aedeagus and parameres are derived from the same pair of primary appendages.

(b) The genitalia are located on the ninth abdominal segment. This is in accordance with the views of Singh Pruthi and George and is also the common position of the genitalia in other orders of the Insecta.

According to Kershaw and Muir, however, the subgenital plates are derived from the appendages of the eighth segment which move posteriorly on to the ninth in the course of development.

In the youngest nymphs examined for this study the subgenital plates were always found to develop in association with the ninth sternite.

(c) The subgenital plates appear to represent the coxites; the aedeagus and parameres together, the telopodites of the ninth segment. This again is in agreement with the views of Singh Pruthi and George. Kershaw and Muir, however, having located the gonopore on the eighth segment, and derived the subgenital plates from the appendages thereof, homologize the latter with the telopodites of the eighth segment and hence with the anterior ovipositor lobes in the female.

It is generally considered that this is not the case, the gonopore being located posteriorly to the ninth sternite and the subgenital plates representing the coxites of this segment.

(ii) The Efferent System.—(a) The gonopore, as in the males of most orders of the Insecta, is posterior to the ninth sternite. Of the previous workers who have studied the development in the Homoptera, Kershaw and Muir alone depart from this conclusion, locating the gonopore posteriorly to the eighth segment.

(b) The efferent system, other than the testes and vasa deferentia proper, is ectodermal in origin.

This is in agreement with Singh Pruthi's conclusions.

In comparing my slides with his figures on Pl. 5(13), I am inclined to think that Singh Pruthi has misinterpreted some of the structures. For example, in figs. 16, 1E, he has labelled as 'ejaculatory ducts' the structures which I consider the accessory glands (cf. figs. 4 and 5, Pl. 27). The accessory glands

are situated nearer to the median line, and the ejaculatory ducts and vasa deferentia more laterally. The situation of the accessory glands nearer to the median line, and their development from the ventral portion of the division of the median duct are constant in all the specimens examined (figs. 5 and 6, 11 and 12, Pl. 27). It is curious that, while Singh Pruthi shows this development in figs. 2 A and 2 B, he does not realize its significance in relation to figs. 1 A-E.

George takes an entirely different view of the matter. He states that the whole of the efferent system, with the exception of the median posterior portion of the ejaculatory duct, is mesodermal in origin. This is in agreement with the earlier work on the development of the efferent system in the Insecta, e.g. Verson and Bisson (18), Wheeler (19). More recent work, such as that of Singh Pruthi and myself (21) on the Coleoptera, tends to the conclusion that, with the exception of the testes and vasa deferentia proper, the efferent system is ectodermal in origin.

Comparing George's plates with my own, I find a distinct resemblance between his fig. 5, Pl. 27, and my fig. 2, Pl. 27. The structures which, however, he interprets as the ampullae of the vasa deferentia, I consider to be the primary divisions of the median ejaculatory duct. No histological difference between the so-called ampullae and the ejaculatory duct can be discerned in George's drawings, nor does he call attention to any such difference in the text. The problem here presented depends on the extent of the original efferent passages which give rise to the testes and vasa deferentia and which I, unfortunately, have been unable to determine. In a nymph in which the ectodermal structures are developed to the extent shown in figs. 1-3, Pl. 27, there are no traces of vasa deferentia as ducts beyond the posterior region of the testes.

The exact extent of the original efferent passage is a point on which there is division of opinion.

Packard (11), Korschelt and Heider (7) hold that they do not extend beyond the posterior border of the seventh sternite, Wheeler (19), Christophers (1), and George (4) that they may even reach into the ninth segment. Furthermore, Korschelt and Heider postulate a progressive shortening of the embryonic genital

strand and its subsequent replacement by secondary structures of ectodermal origin as one line of evolution. Such a state of affairs actually occurs in the Coleoptera (Muir, 8; Singh Pruthi, 14 and 15; Metcalfe, 7a). It is, therefore, not unreasonable to suppose that such a shortening may have taken place in other orders of the Insecta. On the other hand, the extension of the mesodermal vasa deferentia into the ninth segment still requires to be proved, and the tendency of recent research is against this possibility.

(c) The efferent system, other than the testes and vasa deferentia proper, is unpaired in origin. The lateral ejaculatory ducts and accessory glands are derived from the median ejaculatory duct and are hence primarily unpaired.

George, considering the median ejaculatory duct only is unpaired in origin, regards the glands and vasa deferentia as derived from the original paired rudiments.

Singh Pruthi ascribes separate and distinct origins to the median and paired ejaculatory ducts, the accessory glands being constricted from the latter in precisely the same manner as has been described in *Philaenus*. The paired ejaculatory ducts are supposed to arise as paired rudiments, originating near, though not actually opening upon, the eighth segment. These ducts subsequently come into communication posteriorly with the median ejaculatory duct, and anteriorly with the vasa deferentia. The median ejaculatory duct arises as an ectodermal invagination posterior to the ninth sternite. Singh Pruthi's reasoning is inductive rather than deductive: the presence of such a pair of ducts allows him to compare them with the paired terminal ducts of primitive insects found by Wheeler in the Ephemerid *Blasturus* and Palmen in the Ephemeridae (in this case the terminal ducts are described as mesodermal in origin). Furthermore, in his studies in the Coleoptera, Singh Pruthi compares these ducts with the rudiment of the uterus—an ectodermal duct originating posterior to the eighth sternite: a doubtful comparison as the uterus is unpaired in origin.

From the specimens of *Philaenus* examined, there is no reason to suppose that the lateral ejaculatory ducts are separate structures or that they originate otherwise than as the derivatives of the median ejaculatory duct.

Little attention has been paid to Balbiani's conclusions, but as he does not recognize any ectodermal unpaired structures, deriving all from the union of the paired mesodermal ducts, they have little significance or bearing on this study.

At this point I should like to introduce a comparison between the development of the male efferent system in the Coleoptera and in the Hemiptera as typified by *Philaenus*.

In the Coleoptera, the efferent system may be classed under two headings: (1) the ejaculatory duct and its derivatives, (2) the testicular strand and its derivatives. The ejaculatory duct arises as an ectodermal invagination posterior to the ninth sternite. Anteriorly it divides to give rise to a pair of lateral or paired ejaculatory ducts. Accessory glands are present and may arise from the posterior unpaired region of the duct or from its lateral branches. Vesiculae seminales when present arise as dilations either of the main duct or its branches.

The testicular strand is of mesodermal derivation and gives rise to the testes and to a pair of shortened vasa deferentia which rarely extend farther than the fifth segment where they open into the lateral ejaculatory ducts.

A similar state of affairs appears to hold in the Hemiptera—accessory glands and paired ejaculatory ducts being derived from a median ectodermal invagination arising posterior to the ninth segment.

IV. THE FEMALE.

A. Adult Structure.

(i) *The Genitalia*.—The gonopore is situated posterior to the eighth sternite and is bordered by a pair of genital appendages, the anterior or ventral ovipositor lobes. Two pairs of appendages, the dorsal and posterior or lateral ovipositor lobes, are present in association with the ninth sternite.

(ii) *The Efferent System*.—The paired ovaries are situated in the anterior region of the abdomen and lead posteriorly into the paired oviducts. The latter unite to form the uterus or median oviduct which opens posterior to the eighth sternite. Opening into the median oviduct in its dorsal region is a bag-like structure, the spermatheca. The principal accessory

gland is a median structure opening between the dorsal ovipositor lobes posterior to the ninth sternite.

B. Development.

(i) *The Genitalia*.—In the young nymph, two pairs of appendages are clearly discernible, one pair on the eighth, the other on the ninth segments.

As development proceeds a groove appears on each of the appendages of the ninth segment marking off an inner from an outer pair. This groove deepens until two pairs of appendages can be clearly seen, the inner pair being the dorsal ovipositor lobes, the outer the lateral ovipositor lobes (figs. 22, 23, and 27-9, Pl. 28).

The appendages of the eighth segment, or anterior ovipositor lobes, show no secondary division.

(ii) *The Efferent System*.—The origin and development of the efferent system are singularly easy to follow, ectodermal and mesodermal structures being clearly distinguishable.

The ovaries and ducts leading therefrom are derived from the mesoderm. The ovaries lie in the fifth abdominal segment; the oviducts are slender and extend into the sixth segment, where in the early stages of development they end blindly (fig. 16, Pl. 28).

All other parts of the efferent system are ectodermal in origin, and three distinct centres of growth are involved:

(a) Posterior to the seventh segment a duct arises as a wide and thick-walled invagination of the ectodermal layer which divides anteriorly to form a pair of blind-ended ducts (figs. 17-19, Pl. 28). This is the rudiment of the common oviduct.

(b) A second duct is formed by the invagination of the ectoderm posterior to the eighth segment. It extends into the seventh segment, dorsal to the common oviduct, and is the rudiment of the spermatheca (figs. 18-21, Pl. 28).

(c) A third invagination of the ectoderm is present posterior to the ninth segment and extends dorsal to the spermatheca into the eighth segment. This is the rudiment of the main accessory gland (figs. 21-3, Pl. 28). Its opening is situated between the dorsal ovipositor lobes.

In the course of development the original opening of the common oviduct becomes closed over. Its anterior arms grow forward to meet the blind ends of the mesodermal oviducts, the intervening walls breaking down to form a continuous passage from the ovary to the common oviduct.

The spermatheca retains its opening which thus forms the functional gonopore posterior to the eighth segment. Where it overlies the common oviduct in the seventh segment, the two ducts become closely applied to one another. Eventually their intervening walls break down and a common duct is formed, whose dorsal wall is derived from the spermatheca, whose ventral wall is derived from the common oviduct. This composite duct opens to the exterior through the gonopore which lies posteriorly to the eighth segment. The anterior blind end of the spermatheca is not involved in this union, but remains as a dorsal sac opening into the genital duct—the functional spermatheca.

The main accessory gland retains its external opening and does not come into communication with the spermatheca or the common oviduct.

C. Conclusions.

(i) The Genitalia.—(a) The genitalia are paired in origin.
(b) They are derived from two pairs of primary appendages, the one pair situated on the eighth, the other on the ninth segment. The pair on the ninth segment subsequently divides to give rise to two pairs of appendages.

(c) The genitalia are of the simple type directly comparable with the primitive type of Verhoeff (16 and 17), viz. the anterior ovipositors representing the telopodites of the eighth; the dorsal and posterior, the telopodites and coxites respectively of the ninth segment. The dorsal ovipositor lobes are the homologues of the aedeagus and parameres, the posterior ovipositor lobes of the subgenital plates in the male. These conclusions are in complete agreement with George.

(ii) The Efferent System. (a) The gonopore is situated posteriorly to the eighth segment. There is so much variation in the location of the female gonopore that it seems that this opening is not homologous in the different orders. In the

Ephemeroptera there is a pair of openings posterior to the seventh sternite, in the Orthoptera the median opening may lie posteriorly to the seventh sternite (Wheeler 20) or to the eighth (Walker 19), in the Hemiptera and Diptera posteriorly to the eighth, in the Hymenoptera, Coleoptera, and some Lepidoptera posteriorly to the ninth. The male gonopore is constantly posterior to the ninth sternite and is hence only homologous with the female gonopore when this is so situated. It has been suggested by several authors, e.g. Singh Pruthi (15), George (4), Nel (9), that the line of evolution in the female genital system lies in the posterior shifting of the gonopore, and recent research seems to bear out this conclusion.

(b) With the exception of the ovaries and the anterior region of the oviducts which are mesodermal, and derived from the primary efferent passages, the efferent system is ectodermal in origin. This conclusion is supported by the work of Nussbaum (10), Singh Pruthi (15), Jackson (5), George (4), and Metcalfe (7a).

(c) The ectodermal regions of the efferent system are unpaired in origin.

This unpaired condition appears to be primary, and not secondary as Nussbaum, Jackson, and Verson and Bisson concluded. Singh Pruthi and George are also of the opinion that it is the primary condition.

George's account of the development of the ectodermal region of the efferent system in the Homoptera differs in some details from the one given above. According to him, the common oviduct is formed in two parts, the one from an invagination posterior to the seventh segment which also gives rise to the spermatheca, and the other from an ectodermal groove originating on the eighth sternite which subsequently becomes closed over to form a complete tube. These two parts come into communication with each other and open posterior to the eighth segment. The accessory gland originates as an ectodermal invagination posterior to the ninth segment. It appears that the structures which George interprets as the ampullae of the mesodermal oviducts are in reality the anterior divisions of the unpaired oviduct and are hence of ectodermal origin (fig. 17, Pl. 28). Again, the spermatheca is derived, not from the

invagination arising posteriorly to the seventh segment but from that originating posterior to the eighth.

It is a difficult matter to bring into line the homologies of the various ectodermal ducts which go to make up the efferent system, since it appears that structures having the same origin may in different orders have a different function.

For example, in the Coleoptera the common oviduct is derived from two primary invaginations originating posterior to the eighth and ninth sternites respectively. Communication between these ducts is subsequently established, the aperture to the one arising posterior to the eighth being closed over and the gonopore being situated posteriorly to the ninth segment. The functional spermatheca is derived from the invagination arising posteriorly to the ninth segment.

Comparing this with the development in the Homoptera, the so-called spermathecal rudiment of the Coleoptera is homologous with the median accessory gland, the uterine rudiment with the spermathecal rudiment. The unpaired oviduct arising posteriorly to the seventh segment has no duct-like counterpart in the Coleoptera, but in this order the chitinous rod lying ventral to the uterus, and giving attachment to the muscles controlling it, has its origin as an ectodermal invagination posterior to the seventh segment. The invagination grows forward and a solid rod of chitin is secreted by the cells of its wall, its external aperture being thus closed over. This rod, from its position and mode of origin, appears to be the homologue of the unpaired oviduct in the Homoptera which has lost its function as a duct and has assumed a mechanical one.

Again, the median accessory gland and spermathecal rudiment in the Homoptera may be compared with the caecus and uterine rudiments in the Diptera (Christophers).

It appears, therefore, that the various parts of the efferent system are not homologous in the different orders and that no general plan of the female genital system in the Insecta can be devised. There does appear to be, however, a tendency for the gonopore to be shifted posteriorly, and for this posterior shifting to be associated with the suppression of accessory openings to the efferent system and its welding into one main duct.

V. SUMMARY.

1. The genitalia are paired in origin and appear to represent, in the male the coxites and telopodites of the ninth abdominal segment; in the female the telopodites of the eighth, and the coxites and telopodites of the ninth segments.

2. The testes and vasa deferentia, ovaries and oviducts, are paired and mesodermal in origin.

3. The efferent system, other than the testes and vasa deferentia, ovaries and oviducts, is unpaired and ectodermal in origin.

4. The gonopore is serially homologous in the male and female; but is posterior to the ninth segment in the former, and posterior to the eighth segment in the latter.

5. The ejaculatory duct and the median uterus are not strictly homologous, the ejaculatory duct being more comparable with the median accessory gland in the female.

6. There seems to be, in the females of the Insecta, a tendency for the gonopore to be shifted posteriorly.

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EXPLANATION OF PLATES 27 AND 28.

PLATE 27.

Reproductive System of *Philaenus spumarius* L.

The Male.

Figs. 1-3.—Sections through the posterior abdominal segments of a young nymph from posterior-anterior. $\times 200$.

Figs. 4-10.—Sections through the abdomen of an older nymph from anterior-posterior. $\times 140$.

Figs. 11-15.—Sections through the abdomen of an old nymph from anterior-posterior. $\times 90$.

The Female.

Figs. 16-23.—Transverse sections through the abdomen of a young female nymph from anterior-posterior. $\times 105$.

The Male.

Figs. 24-6.

Fig. 24.—Ventral view of the abdomen of a young nymph. $\times 30$.

Figs. 25 and 26.—Growth points in the efferent system.

The Female.

Figs. 27-9.

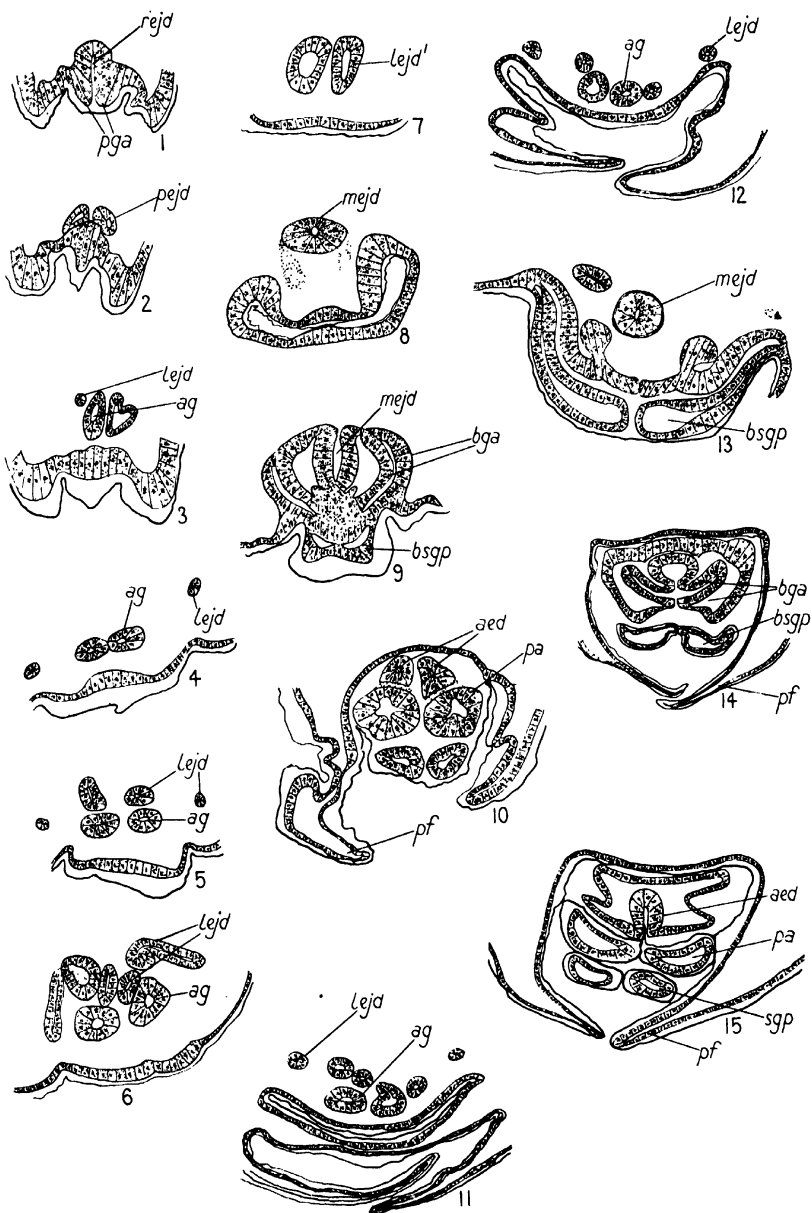
Fig. 27.—Ventral view of the abdomen in an old nymph. $\times 30$.

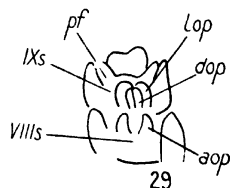
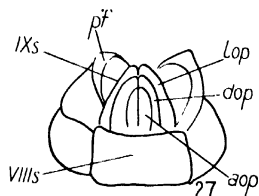
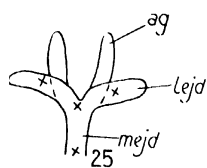
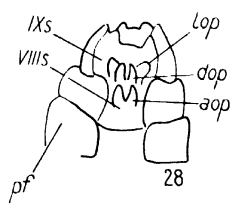
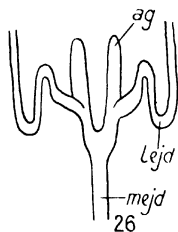
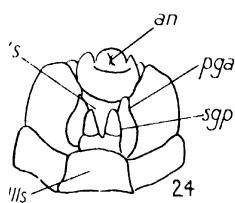
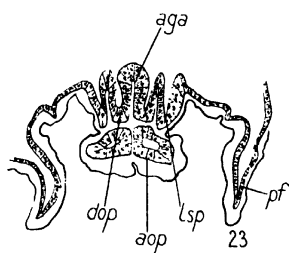
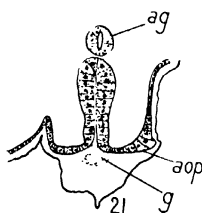
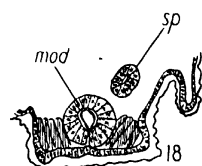
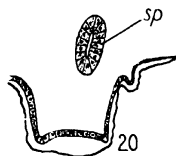
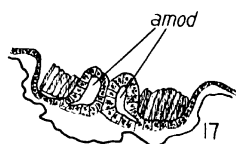
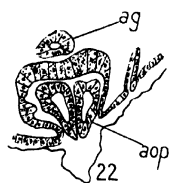
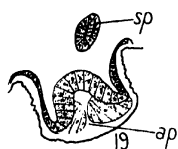
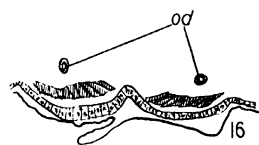
Fig. 28.—Ventral view of the abdomen of a younger stage than fig. 27. $\times 30$.

Fig. 29.—Ventral view of the abdomen in a young nymph. $\times 30$.

LETTERING.

aed, aedeagus; *ag*, accessory gland; *aga*, external opening of accessory gland; *amod*, anterior divisions of median oviduct; *aop*, anterior ovipositor lobes; *ap*, original opening of median oviduct; *bga*, bases of genital appendages (aedeagus and parameres); *bsgp*, bases of subgenital plates; *dop*, dorsal ovipositor lobes; *g*, gonopore; *lejd*, lateral ejaculatory duct; *lejd*¹, lateral ejaculatory duct posterior to origin of accessory gland; *lop*, lateral ovipositor lobes; *mod*, median oviduct; *od*, median oviduct; *pa*, paramere; *pejd*, primary divisions of ejaculatory duct; *pf*, pleural fold; *pqa*, primary genital appendages; *rejd*, rudimentary invagination of ejaculatory duct; *sp*, spermatheca; \times , growth points; *IX s*, ninth segment; *VIII s*, eighth segment.





Notes on *Planaria vitta* Dugès.

By

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With 4 Text-figures.

SPECIMENS of a white planarian found in springs and streams in the Lake District were examined in the Laboratory at Wray Castle, Westmorland. Later, observations were made in Wales on the same species, and collections made for subsequent examination. Numerous animals from both localities have been cut into serial sections, and have been identified as *Planaria vitta* Dugès.

Planaria vitta has been confused with *Planaria albissima* Vejd. Therefore, after a description of the anatomy of *Planaria vitta*, a careful comparison of the details found in this species will be made with those which have been established for *Planaria albissima* by Continental workers. The particular necessity for doing this will be evident in the discussion which follows the descriptive part of this paper.

Planaria vitta is pure white in colour, or sometimes, after feeding, pinkish. The largest specimens measured by the author were 12-13 mm. long, and 2-3 mm. broad. The narrow body has parallel sides for the greater part of its length, but tapers gradually to the rather pointed posterior end.

The margin of the head is uniformly rounded, and completely lacks auricular processes. The eyes are situated far back, and one of the most characteristic external features of *Planaria vitta* is their closeness to the mid-dorsal line.

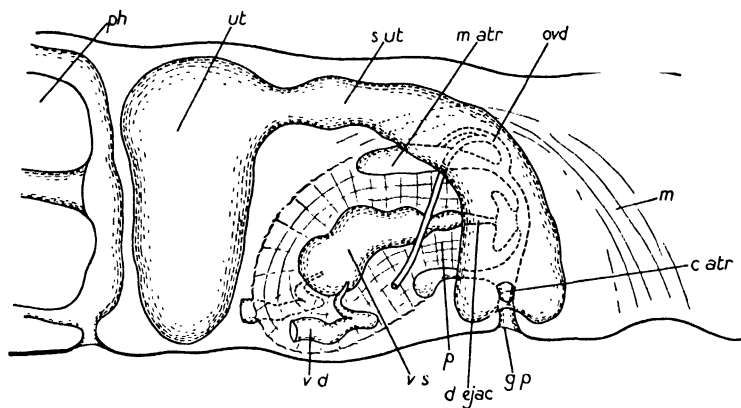
The number of gut diverticulae varies with the size of the animal. The extremes of those examined are: 16, 2(9), 16, and 20, 2(11), 20.

All the larger specimens in the Lake District and Wales have fully developed genital organs, but the finding of anterior and

posterior halves of animals shows that reproduction takes place by transverse fission as well as sexually.

The following description of the internal anatomy is based on series of sections of specimens from both localities. The differences between individuals are very slight, and the general relationships of size and position of the various organs are remarkably constant.

Each animal is, of course, hermaphrodite. The male sexual



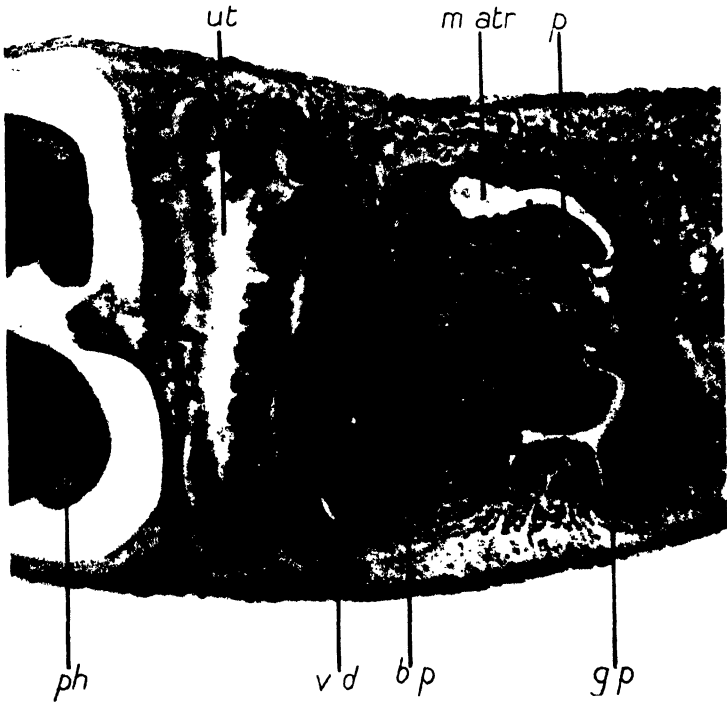
TEXT-FIG. 1.

Diagram of the genitalia of *Planaria vitta*, reconstructed from series of sections. *c. atr.*, common atrium; *d. ejac.*, ductus ejaculatorius; *g. p.*, genital pore; *m.*, muscle which approximates the base of the penis to the genital pore region; *m. atr.*, male atrium; *ovd.*, oviducts; *p.*, penis; *ph.*, pharynx; *s. ut.*, stalk of the uterus; *v. d.*, vas deferens; *v. s.*, vesicula seminalis; *ut.*, uterus.

apparatus consists of testes, vasa deferentia, vesicula seminalis, penis, and male atrium (atrium masculinum). The testes are arranged serially on either side between the gut diverticula. Most of the follicles are large and extend from the dorsal to the ventral surface of the animal, but the smaller ones among them are situated ventrally. Testes are to be found much nearer the posterior end than is normal for Triclad. Some occur even behind the genital mass. Text-fig. 2 shows a testicular follicle just behind the posterior wall of the male atrium.

The twisted vasa deferentia open separately into the vesicula

seminalis, after traversing the musculature of the bulbus penis. Their course through the muscles is not straight, and at one point they make a sharp twist. The vesicula seminalis is a roomy cavity occupying an axial position in the bulbus penis and proximal part of the penis proper. By a gradual narrowing



TEXT-FIG. 2.

Sagittal section of the genitalia of *Planaria vitta*. *b.p.*, bulbus penis. Other lettering as in Text-fig. 1.

posteriorly the vesicula seminalis becomes the wide ductus ejaculatorius, which opens symmetrically at the free end of the penis.

The penis itself is short and blunt. In its substance both longitudinal and circular muscle-fibres are only weakly developed. The end of the penis is of a peculiar and characteristic

shape, which can best be understood by reference to Text-fig. 1. The space between the outer circular fold and the true end of the penis is called by Beauchamp (1926) the 'Cavité préputiale'.

The penis nearly fills the male atrium, of which the surrounding musculature is continuous with that of the bulbus penis. A specially well-developed group of muscle-fibres can be seen in some individuals to extend from the dorsal surface of the



TEXT-FIG. 3.

Sagittal section of the genitalia of *Planaria vitta*, showing a testicular follicle behind the genital mass. *t.*, testis. Other lettering as in Text-fig. 1.

male atrium to the body-muscles ('Hautmuskelschlauch' of the German workers) in the region just posterior to the genital pore.

Although it has not yet been possible to observe animals in a state of sexual activity the function of these muscle-fibres is clear. By contraction they will approximate the base of the penis to the genital-pore region during copulation.

The genital pore is immediately ventral to the extreme posterior part of the male atrium, and the two are connected by a narrow passage. This represents the common atrium (atrium

commune), and with it the posterior part of the stalk of the uterus communicates by way of a relatively small opening.

In this paper the name 'uterus' is used on the understanding that no functional significance is implied. Other names which have been given with the intention of indicating the function of the organ are: 'Drüsenblase, Schalendrüse' (Iijima, 1884; Mattiesen, 1904), 'Receptaculum seminis' (Wilhelmi, 1909), 'Begattungstasche, Gestielter Drüsensac' (Steinmann, 1913), and 'Bursa copulatrix' (Burr, 1912).

In some preparations of *Planaria vitta* spermatophores were observed in the uterus, which proves that its function is the same as in other Triclad. Also a condition was found in one specimen which throws light on the statements of Hallez (1887), and Chichkoff (1892), who maintained that fertilization of the ova took place in the uterus, and that yolk-cells, derived from yolk-glands in other parts of the body, were frequently to be seen there. The prevalent idea at the time was that cocoon formation took place in the uterus.

It has already been proved that the uterus functions as a bursa copulatrix (Burr, 1912), and that fertilization of the ova occurs in the tuba of the oviduct, immediately after they have left the ovary. Further, cocoons are formed not in the uterus, but in that part of the atrium into which the oviducts open.

The explanation of the observations of the two earlier workers is that at certain periods in the reproductive cycle, the cells lining the uterus are shed into its cavity. These were mistaken for yolk-cells, a mistake which is very easily understandable since the two sorts of cells are very similar both in size and as regards their inclusions.

In both structure and position the ovaries and oviducts are quite normal, and show no special deviations from the usual Triclad arrangement. The oviducts do not open into the stalk of the uterus, but into the male atrium. After leaving the ovary each oviduct runs parallel with the edge of the body till it reaches the level of the penis. Here it turns dorsally to unite with its fellow of the opposite side. This union occurs ventrally to the stalk of the uterus, and anterior to its connexion with the common atrium. The unpaired oviduct is short, and leads

posteriorly to open into the posterior wall of the male atrium, not far from the tip of the penis.

It is probable that self-fertilization is prevented by a retention of the spermatozoa in the vasa deferentia until the penis is extended. The mechanism for this would appear to be very like that described in *Bdellocephala punctata* (Ulllyott and Beauchamp, 1931).

In all Triclad systematics the genitalia are most important, and in all cases much of the tissue making up the genital mass is muscular. Standardization of methods for fixing and killing is, therefore, specially desirable, since the different degree of contraction of the muscles, when different reagents are used, produces results which are difficult to correlate and compare in the final preparations.

A routine which has been found valuable is one modified after the recommendations of Steinmann (1913). The animals are killed in Steinmann's fluid, a mixture of one part of strong, commercial, nitric acid, one part of a saturated solution of mercuric chloride in a 5 per cent. sodium-chloride solution, and one part of distilled water. In this solution death is instantaneous and practically no muscular contraction occurs. The animals are then transferred immediately into a quantity of Zenker's fluid. In this they should remain for twenty to thirty minutes, after which they are placed on cotton wool in a large quantity of 95 per cent. alcohol containing a little iodine. This ensures that any precipitate of mercuric chloride is dissolved out. After dehydration the animals are embedded in paraffin wax and then cut into serial sections in the usual way, subsequently being stained and counterstained in haematotylin and eosin.

DISCUSSION.

Carpenter (1926) recorded the occurrence of *Planaria albissima* Vejd. in Wales. This identification was confirmed by Percival and Whitehead (1926), and Carpenter has used the name in three papers (Carpenter 1927 *a* and *b*, and 1928).

According to Continental records, *Planaria albissima* is rare and very restricted in its distribution. It has only been recorded four times, namely, in Bohemia (Vejdovski, 1895), in

Steiermark (Böhmig, 1909), in Bulgaria (Schichkov, 1924), and in the Moselle region (Rémy, 1926). Beauchamp (1926) states that he was supplied by Rémy with the *Planaria albissima* necessary for his comparative work on this species and *Planaria vitta*. Even with the few localities mentioned *Planaria albissima* occurs only intermittently; Böhmig (1909) says: 'Tritt zuweilen in grosser Zahl auf, und verschwindet dann plötzlich.'

Planaria vitta, on the other hand, has been recorded from Bohemia (Vejdovski, 1882; Hahn, 1925), the Odenwald (Lauterborn, 1904) Rügen (Thienemann, 1906 and 1926), the French Jura and the neighbourhood of Tübingen (Böhmig, 1909), the east Pyrenees (Vandel, 1920), Montpellier (Vandel, 1921), Algeria (Gauthier, 1923), Steiermark (Sekera, 1925), Spain (Arndt, 1926), and Cape Finisterre (Beauchamp, 1926).

These facts, together with the discovery of *Planaria vitta* in the Lake District, made it seem desirable to investigate personally the white planarian which had been found in Wales.

A representative number of these animals was collected from various places in the Aberystwyth district mentioned by Carpenter (1928). Some were transported alive in thermos flasks to the Laboratory at Wray Castle, and others were fixed immediately after collection.

Neither in appearance nor in any anatomical characters could the slightest difference be detected between specimens of *Planaria vitta* from the Lake District and the white planarians from Wales. The two are, in fact, identical. A review of the factors which show conclusively that this single English species of white planarian is *Planaria vitta*, and not *Planaria albissima*, is therefore necessary.

Here it must be emphasized that in recent years great steps forward have been made in the systematics of the lesser known freshwater Triclad. All the work on this subject has been done on the Continent, and much has been published in journals which have not a very wide circulation. As a result, many of the older anatomical accounts have been proved to be inaccurate.

This specially concerns the case of *Planaria vitta* which was first accurately described by Vandel, 1921. There is no

doubt that, as Vandel suspected, Vejdovski's sketch of the genitalia of *Planaria vitta*, published by Böhmig (1909), is wrong, and that both the diagram and the description (pp. 158-9) are certainly referable to one of the Dendrocoelids. Beauchamp (1926) published a paper in which *Planaria vitta* and *Planaria albissima* were specifically compared. *Planaria albissima* had previously been described by Vejdovski (1882), Sekera (1888), and Böhmig (1909).

There is, therefore, trustworthy work upon which to base the identification of the English white planarian.

Vejdovski (1895) describes *Planaria albissima* as 'die kleinste Wasserplanarie, von kaum 10 mm. Länge' (p. 208), and Böhmig (1909) gives the length as 8-10 mm.; Böhmig (1909) and Steinmann (1913) give the length of *Planaria vitta* as 10-15 mm., and, as we have seen, specimens found in England measured 12-13 mm.

The head outline and the position of the eyes is shown by Beauchamp (1926) for both species. The representation of *Planaria vitta* agrees with the form of the animals from the Lake District and Wales. Dugès, who first described *Planaria vitta*, speaks of the eyes as 'fort rapprochés l'un de l'autre', and mentions 'L'absence des angles ou sub-auricules de la tête'. Vejdovski (1895), Sekera (1888), and Böhmig (1909) have nothing to add to this, but Steinmann (1913) says: 'Augen klein; ihr gegenseitiger Abstand beträgt ungefähr ein Fünftel der Körperbreite.'

The state of affairs in *Planaria albissima* is quite different. Vejdovski (1895) says that the eyes are 'weit von einander entfernt' (p. 208), but Böhmig (1909) seems less certain, and Steinmann (1913) gives the diagnosis: 'Zwei Augen nicht sehr nahe beieinander: ihr Abstand soll etwas schwanken.'

The schemata for the gut diverticulae according to various authors are given in the table:

<i>Author.</i>	<i>Planaria vitta.</i>	<i>Planaria albissima.</i>
Böhmig (1909)	—	17-20, 2(6-8), 17-20
Steinmann (1913)	18-19, 2(11), 18-19	17-20, 2(6-8), 17-20
Beauchamp (1926)	25-30, 2(10-12), 25-30	18, 2(10-12), 18

This means that no importance can be attached to the gut diverticula for the purposes of identification. Variations certainly occur with the age of the animal, and probably with locality, too.

As in all systematic work on the Triclad, the genitalia are relied on for providing the constant diagnostic characters of the particular species. We have already seen that the description of the genitalia of *Planaria vitta* given by Böhmig (1909) is wrong, and that accurate accounts are given by Vandel (1921) and by Beauchamp (1926). The anatomy of the animals collected in England agrees so accurately with these accounts, and shows so many differences from *Planaria albissima*, that there is no choice but to conclude that these white planarians are *Planaria vitta*.

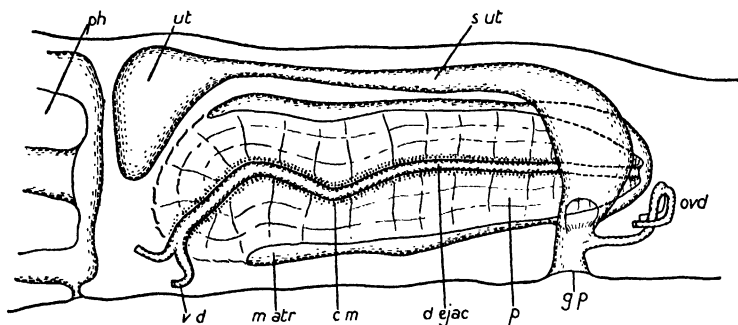
In the first place the uterus of *Planaria albissima* is 'von geringer Grösse' (Böhmig, 1909, p. 165), and the anterior part of the stalk of the uterus is long and narrow while the posterior part is muscular and wide. Also in this species the oviducts unite posteriorly to the connexion between the stalk of the uterus with the common atrium, and open into the atrium from behind the genital mass.

The position of the union of the oviducts in relation to the stalk of the uterus has not been sufficiently emphasized in Triclad literature, but the fundamental difference between the two types of condition referred to in this paper can be seen by comparing Text-fig. 1 with Text-fig. 2.

From this consideration of the female genitalia alone, it is evident that the animal recorded in England can hardly be *Planaria albissima*. The male genitalia provide even more striking evidence against this assumption. Böhmig (1909) describes this species as follows: 'Der Kopulationapparat bietet einige charakteristische Eigentümlichkeiten. Der zylindrische, stumpf zugespitzte Penis steht an Grösse wenig hinter der Pharynx zurück. Die Vasa deferentia einigen sich sofort bei ihrem Eintritt in den sehr kleinen Penisbulbus zum Ductus ejaculatorius der in ganzer Länge von einer dicken Schicht von Ringsmuskeln umgeben wird. Von noch grösserer Mächtigkeit ist die äussere Ringsmuskelschicht des Penis . . .' (p. 165).

Beauchamp (1926) agrees in the main points with Böhmig and says that the penis is four times as long as its width at the base. He finds that the male atrium extends considerably posterior to the genital pore, and in his diagram the unpaired oviduct does not open so close to the genital pore as Böhmig indicates.

From the descriptions it is clear that, if the English white planarian were *Planaria albissima*, it should have



TEXT-FIG. 4.

Diagram of the genitalia of *Planaria albissima*, after Böhmig and Beauchamp. *c.m.*, circular muscles of the penis. Other lettering as in Text-fig. 1.

genitalia with the oviducts uniting posterior to the genital mass, a large, long, and muscular penis with a very small bulbous and no special form of the distal end. Further, the vasa deferentia should unite immediately to form the ductus ejaculatorius, so that no vesicula seminalis should be present. Also the testes should be confined to the more anterior parts of the body. As we have seen, this is not the case—on the contrary all the anatomical features indicate that the animal is *Planaria vitta*.

Ecological considerations emphasize the correctness of this view. *Planaria albissima* has only been recorded from a relatively restricted region round the central European mountains, whereas *Planaria vitta* is known from North Africa to Rügen and Cape Finisterre.

SUMMARY.

A white planarian found in the streams of the Lake District is shown to be *Planaria vitta* Dugès. Previously Carpenter (1926) has identified a white planarian common in streams in Wales as *Planaria albissima* Vejd. A reinvestigation of this animal shows that it is identical with the one from the Lake District, and is, therefore, *Planaria vitta*.

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The Development of the Ectodermal Nerve Net in the Buds of Hydra.

By

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With Plates 29-31 and 1 Text-figure.

INTRODUCTION.

IN 1890, in his work on *Hydra*, Schneider brought together the main facts from the then existing literature concerning the nervous system of *Hydra*. In summarizing his comparisons and findings he came to the following conclusions: 'Die Ganglienzellen besitzen einen kleinen Kern ohne Nucleolus, wenig Protoplasma um denselben und lange, sich verstellende, bei Behandlung mit Essigsäure varicose Ausläufer. Letztere verbinden sich untereinander, mit den Epithelmuskelzellen und wahrscheinlich auch mit Nesselzellen' (pp. 361-2). Also, 'Die Ganglienzellen entstehen aus den indifferenten Zellen durch Ausnutzung des centralen Theiles der Zelle zu Gunsten des peripheren (ringförmigen), der sich halbmondförmig auseinander legt und in die Ausläufer auswächst. Der Kern wird kleiner, streckt sich in den meisten Fällen und verliert seinen Nucleolus' (p. 362). Schneider was also of the opinion that the cnidoblasts with their cnidocils were in some manner sensory in nature and in some way connected with the nerve net.

However, Hadži (1909) found the true sensory cells of the ectoderm and the endoderm: he demonstrated that those of the ectoderm are in direct connexion with the nerve net. Hadži also showed in his work that the cnidoblasts are not of a sensory nature, as Schneider had maintained, but that the (probable) connexion with the nerve net is an artifact. He corroborated the findings of Schneider in that he found the nerve net to be most diffuse on the 'Magenteil', and that the neural processes

from the ganglion cells run between the epithelio-muscular cells (see fig. 25, Pl. 31).

Marshall (1923), who made quite an extensive study of the nervous system of *Hydra*, was not quite able to confirm all the findings of Hadži.

By using specially prepared and stained sections of adult *Hydra* one is able, in some degree, to identify the ganglion cells and their processes: but these same methods when applied to developing buds are almost worthless as to the results which they produce. Since the writer wished to make a study of the development of the nerve net in the buds of *Hydra* it was found to be imperative that he employ some other methods than those used by previous workers. Schneider (1890), it was found, had used a dead maceration method; Miyashima (1898) does not indicate which species of *Hydra* he used in his work, but his method of demonstrating the nerve net with methylene blue was tried; however, it did not work well enough to justify continuation of experimentation. Hadži (1909) used both vital staining with methylene blue in distilled water and specially stained and prepared sections in his study of the adult *Hydra*; but the methods of all these workers, when applied to the developing buds, revealed no facts of any value. Because of this, it became necessary to experiment at length with methods of vital staining until a method was found which would stain the nerve net of the parent *Hydra*, and at the same time stain the indifferent cells of the bud which were being elaborated into ganglion and sensory cells of the developing nerve net in the bud. The method which finally proved most successful was a modification of the Rongalit white method as developed by Unna, and so successfully used by Heider (1927) in his work on the nervous elements of the Ctenophores.

MATERIAL AND METHODS.

Chlorohydra viridissima Schulze and *Pelmato-hydra oligactis* Pallas (Schulze) were used in this study. These *Hydra* were collected from the pools and streams near Ljubljana and brought to the laboratory where they were placed in large aquariums along with various water-plants. Food was

supplied at needed intervals, consisting mostly of Copepods. At regular periods the aquariums were radiated with an ultra-violet light in an attempt to prevent the growth of bacteria; under these conditions the Hydra budded all winter, thus supplying adequate material for study.

A compound, binocular microscope, equipped with a $97\times$ oil-immersion objective, and $10\times$ and $15\times$ eye-pieces, was used in this study. Drawings were made with a camera lucida at various magnifications.

The method of preparing the stain is as follows: to 100 c.c. of 0.5 per cent. methylene blue solution in distilled water add three drops of 25 per cent. HCl. This solution is thoroughly mixed and filtered. To 10 c.c. of this mixture add 2 c.c. of a 15 per cent. Rongalit solution in distilled water. The mixture, methylene blue HCl Rongalit, is now placed in a beaker and gently warmed over the flame of an alcohol lamp or gas-jet. Never should this compound be allowed to come to the boiling-point. While being warmed it should be observed very carefully and stirred constantly. Eventually, the deep-blue colour of the solution begins to slowly change to a deep, dirty green. At this point the solution should be removed from the flame and the stirring continued; in a few minutes the solution becomes almost clear in colour and contains a yellowish precipitate. At this stage the solution should be set aside to cool, after which it is filtered into a dropping bottle and allowed to stand from 24 to 36 hours before being used. This solution is good for 8 or 10 days.

The method for application to green Hydra is as follows: the Hydra are placed in a small glass dish holding about 30–40 c.c. of filtered water from the aquarium in which they have been living. To this dish one adds from 1 c.c. to 2 c.c. of the stain: the water at first turns to a light, milky-blue colour which, in time, becomes deep blue. The Hydra expand and contract normally in this solution. The staining of the nerve net has its beginning at the bases of the tentacles and in the region surrounding the oral opening; proceeding slowly down the body, the solution stains the nerve-cells of the basal disk at the end of about 50 minutes. For studying the nerve net of the

tentacle-tips it is necessary to place the *Hydra* in a few drops of water from the aquarium, under a cover-glass; some of the stain is then drawn under the glass by means of a piece of filter paper; after a few minutes the ganglion and sensory cells of the tentacles are so stained that they may easily be studied.

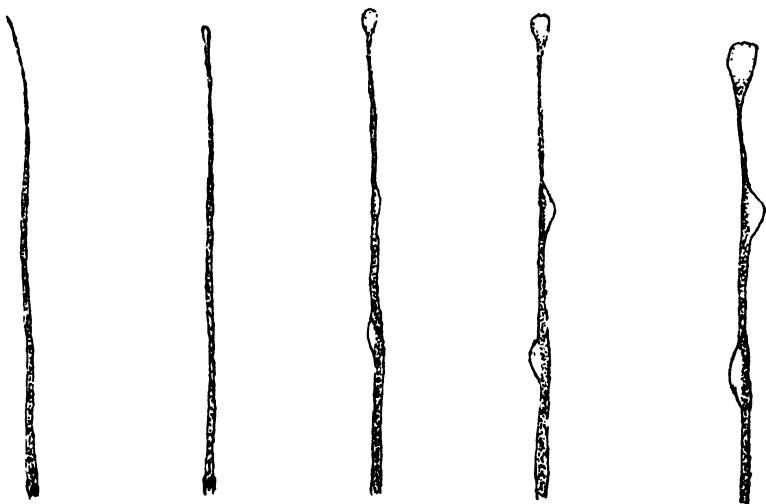
The method for application of this stain to brown *Hydra* differs somewhat from that used with green *Hydra*. The *Hydra* are placed in 30–40 c.c. of slightly alkaline tap-water; to this one adds about $\frac{1}{2}$ c.c. of stain. After about one half-hour the nerve-cells are stained enough for study.

It is to be noted in passing that the nervous elements of green *Hydra* hold their colour for long periods of time after the *Hydra* is placed under the cover-glass, and that in brown *Hydra* the colour fades in from 40 to 50 minutes after being pressed between the slide and the cover-glass. This difference, I am led to believe, is caused by the presence of the simbiotic zoochlorellae in the green *Hydra*, which apparently continue the production of oxygen after the *Hydra* is dead.

The action of the stain on the nervous elements is best followed by placing a brown *Hydra* with a bud under a cover-glass, flooding it with a stain solution and waiting until the stain begins to take effect. By careful observation one may find a nerve-cell with a developing process which is just beginning to take up the stain; keeping the cell under observation one sees, in about ten minutes after the stain begins to have effect, a small bubble begin to form on the end of the process. This bubble slowly increases in size (see Text-fig. 1). Later, small blister-like bubbles form on the sides of the process, and at the place where the process divides. Eventually, these blister-like bubbles arise from the ganglion or sensory cell itself. This action of the stain suggests that the tip is the most sensitive part of the developing process to foreign chemicals, and that these processes are developing and moving outward from the nerve-cells like long pseudopods, as maintained by Plate (1922).

Light apparently has no effect upon the working of the stain, as it has been found to work in semi or total darkness. It is true that, after long periods of time in the stain, other elements of the *Hydra*, such as interstitial cells, nematocysts,

epithelio nuclei, &c., are stained; but their colour can in no-wise be confused with that of the nerve-cells which are stained a deep violet blue. When one is studying the nerve-cells of the basal disc it is necessary to see that the *Hydra* do not attach themselves to the bottom of the dish, as it has been observed that the *Hydra* which were so attached take the stain very poorly or indifferently in the nerve-cells of the basal region.



TEXT-FIG. 1.

The changes in the processes of ganglion cells of *Hydra viridis* over a period of 35 minutes. $\times 2700$.

THE ORIGIN OF THE NERVE NET OF THE BUD.

The origin and formation of the bud has been studied by Tannreuther (1908), Gelei (1925), Kleinenberg (1872), and Hadži (1909, 1911, 1913, 1914), and although they disagree slightly on several points as to causative agent, method of development, and migration or non-migration of the interstitial cells through the mesoglea, they all agree and observe that prior to bud-formation there is a great increase in the number of the ectodermal, interstitial cells at the point where the bud will be formed.

Among these ectodermal, interstitial cells are cells which will

later be elaborated into cnidoblasts, ganglion, and sensory cells of the ectoderm. At an early period the cnidoblast cells already contain the anlage of the nematocyst; but the cells which are to be elaborated into ganglion and sensory cells of the ectodermal nerve net remain undifferentiated until the period in the development of the bud arrives at which the tentacle area first makes its appearance. At this period the interstitial cells of various sizes, which are to be elaborated into ganglion cells, begin to send out processes between the epithelio-muscular cells of the newly forming ectoderm. As further development of the tentacles and mouth region takes place, and the tentacles begin their outward growth, they carry with them the ganglion cells and sensory cells which, prior to this period, have already formed a small area of nerve net by the growing together of the processes from the ganglion and sensory cells. When the tentacles have about completed their growth there begins a wave of development in the nerve net which starts at the bases of the tentacles and sweeps downward over the body of the bud.¹ In front of this wave of development one finds the various change-over forms of the interstitial cells to ganglion and sensory cells (see Pl. 31). When the bud has nearly reached maturity and the ectodermal cells of the basal disk have taken on their characteristic form and appearance, it is found that the nerve net is complete in this region with the exception of the centre; in this central area the nerve net develops during the first day after the bud has been freed from the parent.

There seems to be no rule as to which of the interstitial cells shall be elaborated into nerve-net, ganglion cells, since interstitial cells of various sizes can be found at various stages of sending out processes. There is also apparently no rule as to the number of processes which may develop on a ganglion cell,

¹ Wolff (1903) maintains that the cnidoblasts are in direct connexion with the nerve net; but I have been entirely unable to confirm his findings. When one studies the nerve net of the tentacles quite closely he finds that the processes from the ganglion cells do not divide into enough small branches to innervate the cnidoblasts of a battery, and that the ganglion-cell processes, in most cases, run at quite a distance from where they should be if there were any connexion with the nematocyst through the stalk of the cnidoblast.

and no rule as to how long these may become (see fig. 15, Pl. 29). The directions in which the processes run depend a great deal upon how the epithelio-muscular cells of the ectoderm are oriented since, as Hadži has shown, these processes from the ganglion cells run between the epithelio-muscular cells. The processes from the ganglion cells, as they advance, often divide and subdivide, so that they gradually become smaller and smaller; they become so small, in fact, that many of them are barely visible (see figs. 16, 17, and 18, Pl. 29). Occasionally, especially in the bipolar type found most frequently at the bases of the tentacles, one finds these processes to be very long and not dividing (see fig. 11, Pl. 29). Again, other processes grow towards the surface of the ectodermal layer where they end in the form of small bubbles between the outer ends of the epithelio-muscular cells. The processes which thus grow towards the surface may arise from the division of a process, or from the ganglion cell itself (see fig. 10, Pl. 29, and fig. 20, Pl. 30). The exact nature of these endings is not known, but the author is of the opinion that they are sensory in nature; they are most often found in the region where the fewest sensory cells are found, namely, the so-called stalk region.

The development of the sensory cells takes place along with the development of the ganglion cells. One first finds the interstitial cells, which are being elaborated into sensory cells, on the area around the mouth and on the developing tentacles; they are found, in the majority of cases, protruding through the surface of the epithelio-muscular cells. Occasionally they are found between the epithelio-muscular cells. Regardless of their location, they reach to the ectodermal surface and from their proximal ends send out typical processes which eventually find and coalesce with the processes of the ganglion cells (see figs. 19, 22, and 24, Pl. 30). The interstitial cells which are elaborated into sensory cells cannot be differentiated from the interstitial cells which are being, or are to be, elaborated into ganglion cells except by their location and later development. The nucleus has the same characteristic appearance as that of the ganglion cell except that in the later stages of development the nucleus is rather long and drawn out; this condition is most often noted

when the sensory cell is developing between two epithelio-muscular cells. The sensory cells which develop under and protrude through the exterior surface of the epithelio-muscular cells are usually shorter and thicker; and though they lie contiguous to many other interstitial cells these sensory cells send their processes down between them to the region where the ganglion-cell processes are found (see fig. 22, Pl. 30). The distal end of the sensory cell, upon reaching the exterior surface, develops the characteristic sensory hair described by Hadži (1909); these hairs are found to vary in number from one to five.

The formation of the nerve net takes place by the growing together of the processes from the ganglion cells: these processes advance between the epithelio-muscular cells until they meet other processes from ganglion cells with which they fuse. Other processes from the ganglion cells grow out in various directions and end among the muscular processes of the epithelio-muscular cells. These nerve endings on the muscular prolongations of the ecto-epithelio-muscular cells are entirely undifferentiated as far as can be ascertained microscopically, and it appears that the enervation of these muscular processes is only through contact with the processes from the ganglion cells. The processes which form the nerve net are usually longer than the free-ending processes. There may be two or three processes which connect to those from other ganglion cells, and they are not, as a usual thing, so much branched as the free-ending processes which, as a rule, develop much more slowly than the connecting processes. It is true that in many cases one finds ganglion cells which have many processes radiating from them and which, apparently, have no connexion with the nerve net as a whole; but it can be safely assumed that these have formerly been in connexion with the nerve net but have had their connecting processes torn loose through pressure from the cover-glass and the gradual spreading of the tissues. Upon many occasions, especially when working with brown *Hydra*, I have been able to observe a perfect net under the epithelium of the *Hydra*; this same preparation, from 20 to 30 minutes later, showed only single nerve elements with no connexion between them, the connexions having been broken by pressure from the cover-glass which was

gradually lowered as the water evaporated from around its edges.

The existence of a continuous nerve net is very obvious and has been described in detail by Schneider (1890), Hadži (1909), and Marshall (1923); however, Bozler (1927) maintains that there is no continuity but only contact. The author will bring together, in the near future in another paper, certain relevant facts and findings by which he hopes to show more clearly that continuity definitely exists among the cells of the nerve net of the ectoderm of *Hydra*.

DISCUSSION.

That the bud of *Hydra* does not inherit its nervous system from the mother *Hydra* is quite obvious. By vital staining methods I have been able to determine that the bud area at its earliest stage is served by one, and sometimes two, already-developed ganglion cells of the mother animal; very often this area contains no ganglion or sensory cells but is innervated only by ganglion-cell processes from distantly placed ganglion cells.¹ The fate of the ganglion and sensory cells which do lie within the area where a bud will develop is very difficult to determine, since one finds them but rarely on the developing bud; while at the same time one finds many interstitial cells which are being elaborated into ganglion and sensory cells. The origin of the ectodermal nerve net is from the interstitial cells which are carried out by the developing bud tissues and is not inherited from the parent animal, nor is it developed from the few, ready-formed, ganglion or sensory cells which might lie within the area where the bud will develop.

Efforts to determine which of the interstitial cells would be elaborated into nerve-net cells were fruitless, as the indifferent interstitial cells do not take vital stains when the stain is properly applied. When the proper precautions are exercised one finds that only the nerve-net cells and cnidoblasts of the

¹ This is further evidenced by the fact that some bud areas contract when stimulated, while others, though also stimulated, do not; so that the contraction depends upon whether or not ganglion cells are present in these areas.

parent Hydra take up the stain, and that the future nerve-net cells of the bud are coloured only after they have begun the development of the ganglion processes which will connect them or innervate the epithelio-muscular cells. Why this physiological difference exists is difficult to say, since when one studies these vitally stained preparations he continuously finds unstained, interstitial cells lying side by side with vitally stained, interstitial cells which have the first anlage of a nervous process. Measurements of cell size and nuclear size are found to be approximately the same among such groups; where and when this differentiation as to ability to take up a vital stain originates, the author believes, remains as yet unsolved.

It is true that after long periods of time the indifferent interstitial cells take up some of the vital stain, but with an entirely different shade of colour. This, I believe, argues for the fact that the future nerve-net cells have already differentiated physiologically, since they have previously taken up the vital stain with a totally different shade of colour, namely, bluish-violet; while the indifferent interstitial cells never take on more than a dirty-blue shade.

The wave of development which moves downward from the head pole towards the foot pole does not always, apparently, complete all of the connexions between the ganglion cells and the sensory cells so as to form a complete net, as one finds, even some days after the young Hydra is cast off, that some of the ganglion cells have all free-ending processes. But I believe, from my observations, that the majority of these connexions are made during this downward wave of development and that these others are later differentiations made after the Hydra has begun to increase in size; since when one studies vitally stained Hydra, which are known to be adult Hydra, he finds none of these development forms.

Hadži (1909) is of the opinion that the more deeply placed, free-ending processes from the ganglion cells are motor in nature, as he, by maceration methods, found them to be connected to the muscular prolongations of the ectodermal, epithelio-muscular cells. He is further of the opinion that the free-ending processes which reach the outer surface of the ectodermal epithelium are

sensory in nature. In my study of vitally stained preparations I have not been able to confirm the findings of Hadži as to the free-ending processes running to the muscles, since these muscular prolongations remain entirely colourless. On the other hand, however, I have been able to confirm his findings in macerated preparations prepared after Schneider's methods; also in every vitally stained preparation I have been able to confirm Hadži's findings as to the (as he describes) sensory processes which end on the ectodermal surface. One can, I believe, safely say that the longer, larger processes which connect the ganglion cells are distinctly sensory in nature. One sees then, if the above is true, that in *Hydra* are to be found ganglion cells which produce both motor and sensory processes. My observations of the development of these processes of the ganglion cells has led me to believe that the long, connecting processes ('Hauptfortsätze' of Hadži) develop first, and that the motor processes develop later, as Hadži has shown, as the ganglion cells which lie at the bases of the tentacles very often have only long (sensory), connecting processes and very few, if any, branchings.

Many observations of the developing nerve-cells in the basal region of the tentacles show that these processes become very long before dividing or before any other processes arise from the ganglion cell itself. The development of motor processes would then appear as a secondary development after the formation of the nerve net of the *Hydra*. This condition, namely, of many sensory cells and of ganglion cells with long, sensory, connecting processes and few, free-ending processes, is also found in the basal disk and lends further weight to the argument that these connecting processes are sensory and not motor in nature; since it is already well known that the basal disk is a region of very little muscular movement but rather a region of great sensibility.

Schneider (1890) observed in his work that the nerve-cells arise from interstitial cells, and he pictures the various change-over forms from undifferentiated, interstitial cells to the ganglion cells with long processes (see Schneider, *Tafel XVIII*, fig. 29). However, I am of the opinion that the cells, pictured by him

as the earlier stages of the development, are not future nervous elements, but the cnidoblast cells of various sizes which have lost their nematocysts. I have been able to find many forms such as those he describes and pictures and have been able in all cases to definitely identify them as cnidoblasts by their nuclear measurements, by their colour, and by their location, as well as by the presence of a clear cavity in their cytoplasm where the nematocyst was formerly located. The latter-development forms pictured by Schneider are very similar to some of the forms which I have been able to identify.

Parker (1919) wrote as follows concerning the development of nerve nets: 'In animals in which nerve nets prevail, the lower invertebrates, the embryonic cells that give rise to their protoneurones are in the course of their development near neighbours so that the intimate relations which they bear to one another in the final net may be after all an expression of that closeness of relation that has existed between them from their embryonic states onward. It is not impossible that protoneurones that are united with each other in the nerve net retain their strands of connexion from embryonic stages when in the course of cell division they were really never entirely separated.'

It is true that in the buds of *Hydra* the embryonically possible nerve-cells (i.e. undifferentiated, interstitial cells) do lie in close contact; but it appears that those which are later developed into nervous elements lie quite distant from one another, in fact so far apart that one can preclude the possibility of there being connecting strands between them which might later be elaborated into nervous processes (see fig. 27, Pl. 31).

SUMMARY.

1. The interstitial cells are multiplied at the place where a bud will originate.
2. The buds of *Hydra* develop a nerve net from interstitial cells.
3. The future nerve-net cells remain undifferentiated until a period just prior to the first appearance of tentacle anlage.
4. The formation of the nerve net takes place by the growing together of the processes which arise from the ganglion and

sensory cells. These processes advance in a pseudopod-like manner between the epithelio-muscular cells.

5. The development of the nerve net sweeps downwards from the head pole towards the foot pole of the young *Hydra*.

6. Interstitial cells of various sizes may be elaborated into ganglion or sensory cells.

7. It appears that the long, connecting, sensory processes develop first and that the short, free-ending, motor processes develop later.

8. The great distance that the developing nerve elements lie from each other precludes the possibility of there being protoplasmic strands between them which might be elaborated into nerve processes.

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EXPLANATION OF PLATES 29, 30 AND 31.

PLATE 29.

Figs. 1, 2, 4, and 7.—Developing ganglion cells from basal region of tentacle of *Hydra viridis*. $\times 1,200$.

Figs. 3, 5, 6, and 8.—Developing ganglion cells from ‘magenteil’ of *Hydra viridis*. $\times 1,200$.

Fig. 9.—Two ganglion cells of unequal size. From region of tentacle base; seen from the side; mature *Hydra viridis*. $\times 700$.

Fig. 10.—Ganglion cell with two sensory processes which end between the ecto-epithelio-muscular cells. $\times 750$.

Fig. 11.—Bipolar ganglion cell from base of tentacle of *Hydra fusca*. $\times 780$.

Fig. 12.—Two developing ganglion cells from bud of *Hydra viridis*. $\times 700$.

Fig. 13.—Section of nerve net from mature *Hydra viridis*. $\times 780$.

Fig. 14.—Developing ganglion cell with one divided process and the starting of another branched process. From *Hydra viridis* bud. $\times 1,200$.

Fig. 15.—Ganglion cell from *Hydra viridis* bud. $\times 780$.

Fig. 16.—Developing ganglion cell from bud of *Hydra viridis*. $\times 1,200$.

Fig. 17.—Multipolar ganglion cell from mature *Hydra fusca*. $\times 700$.

Fig. 18.—Developing ganglion cell from bud of *Hydra fusca*. $\times 1,200$.

PLATE 30.

Fig. 19.—Developing sensory cell which lies between two ecto-epithelio-muscular cells. $\times 500$.

Fig. 20.—Sensory process arising from ganglion cell which ends between ecto-epithelio-muscular cells. $\times 500$.

Fig. 21.—Sensory cell protruding through ecto-epithelio-muscular cell of ectoderm of mature *Hydra fusca*. $\times 600$.

Fig. 22.—Developing sensory cell found under an ecto-epithelio-muscular cell. Contiguous to it are seen nine developing cnidoblasts. $\times 500$.

Fig. 23.—Two ganglion cells of different size from the nerve net of mature *Hydra fusca*. $\times 650$.

Fig. 24.—A still earlier stage of a developing sensory cell than that shown in fig. 19. $\times 500$.

PLATE 31.

Fig. 25.—The processes from the ganglion cells run between the epithelio-muscular cells of the ectoderm. Camera lucida drawing. $\times 400$.

Fig. 26.—The location of the ganglion cells on a nearly mature bud of *Hydra fusca*. Slightly diagrammatic.

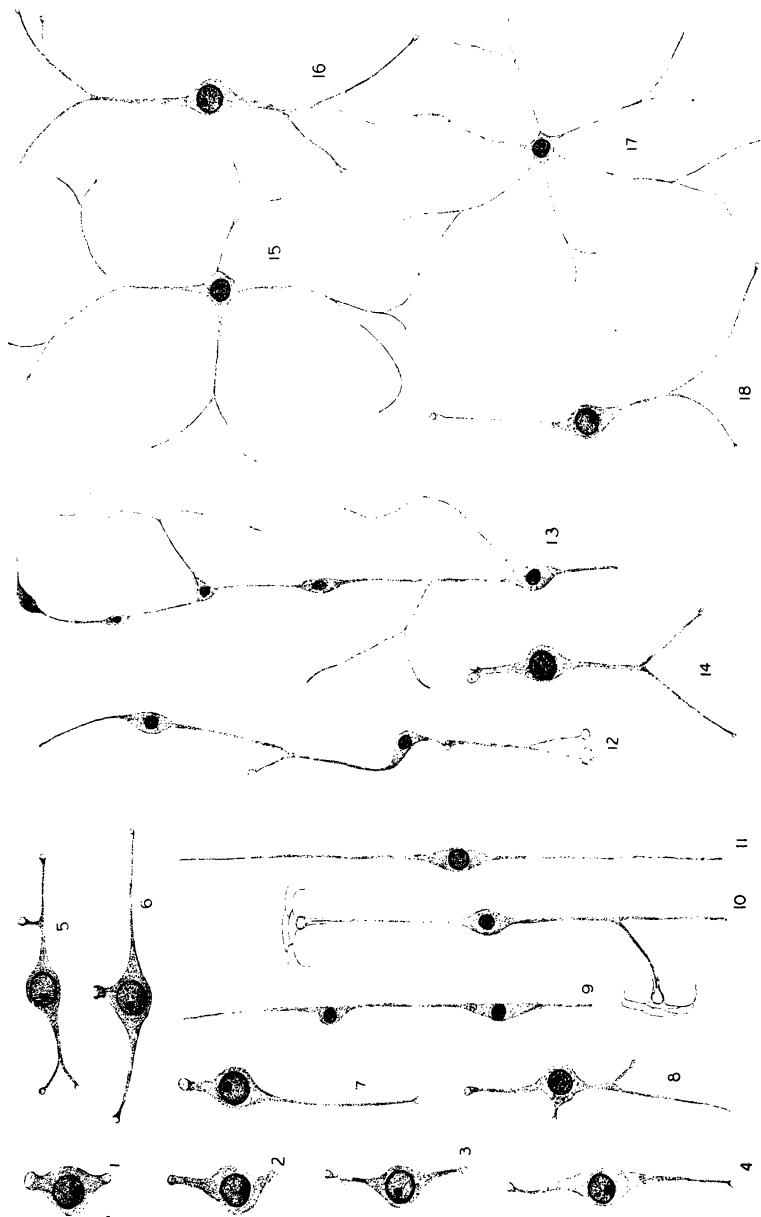
Fig. 27.—Group of interstitial cells from *Hydra fusca*:

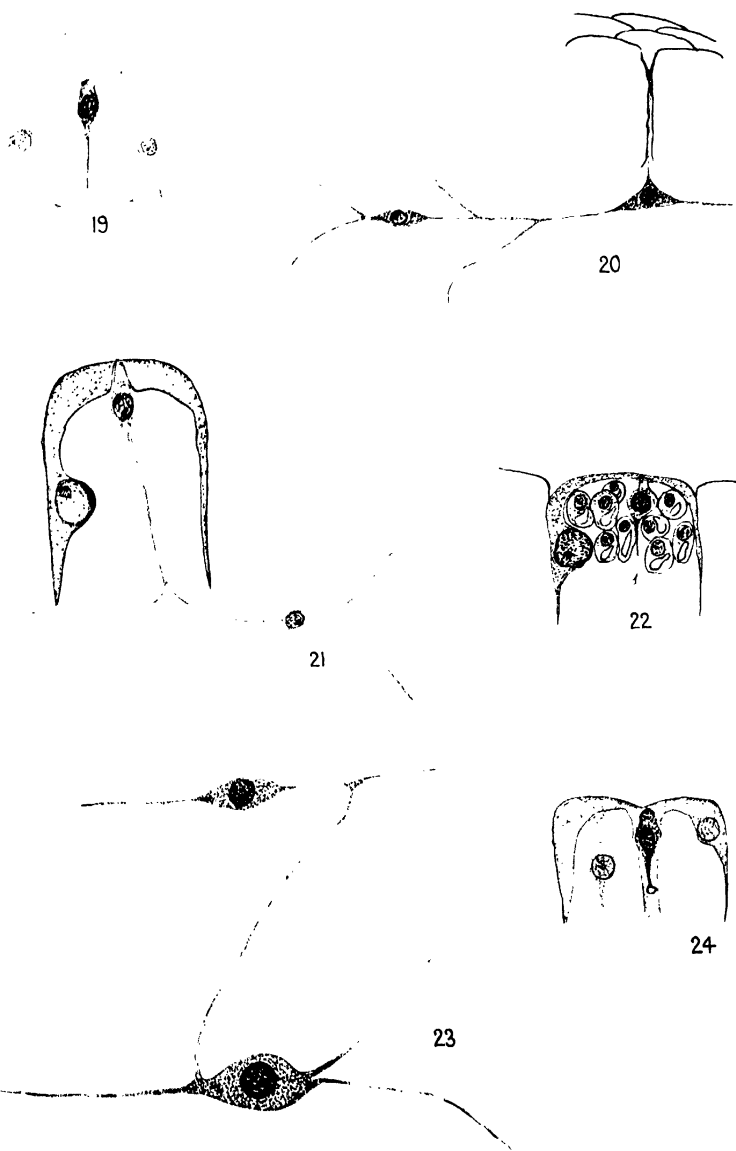
Black—undifferentiated, interstitial cells.

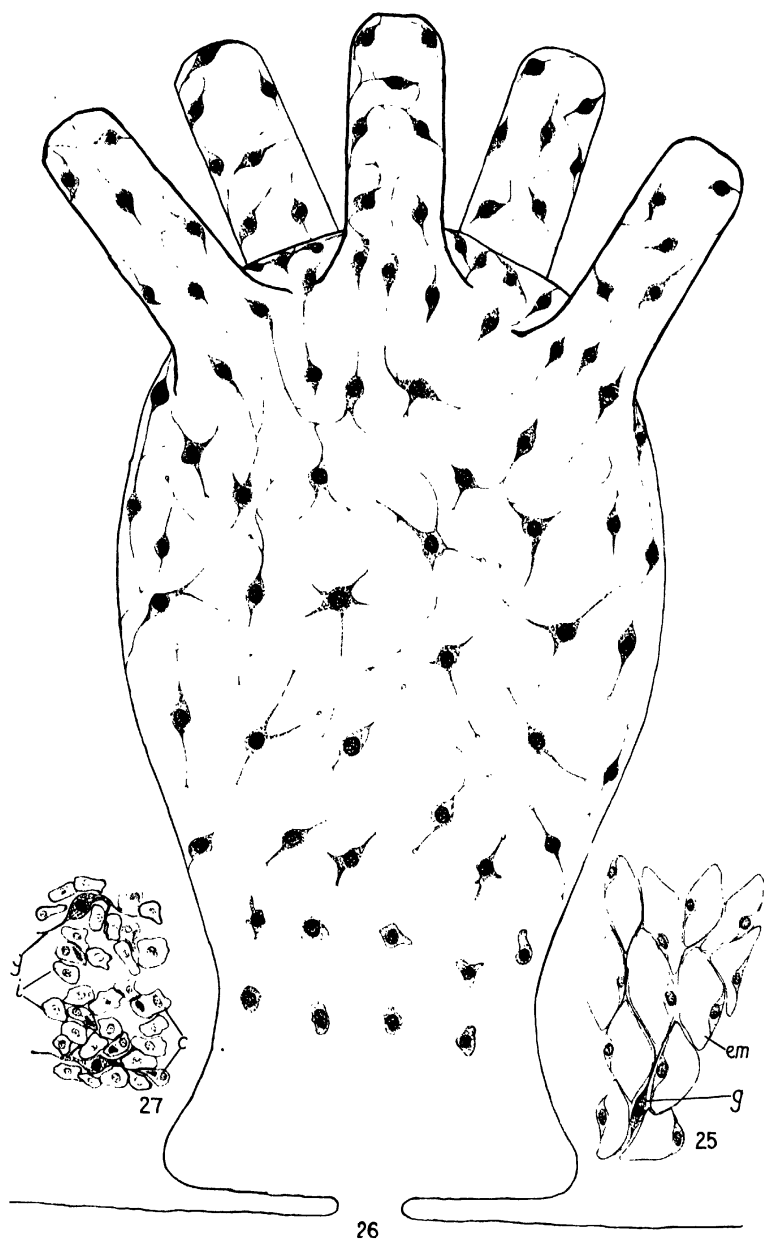
Red—developing cnidoblasts.

Blue—developing ganglion cells.

Distance between ganglion cells—78 microns. $\times 550$.







The Effect of Gamma-Ray Irradiation upon the Growth of a Protozoon, *Bodo caudatus*.

By

Muriel Robertson,

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With 5 Tables and 4 Graphs.

INTRODUCTION.

THIS paper comprises an account of the effect of gamma-ray irradiation upon the growth of the protozoon *Bodo caudatus*, a small biflagellate which feeds upon bacteria. This organism possesses certain advantages as an object of study, since its growth can be controlled; the cells can be counted and measured; and the irradiated strains can be kept alive and tested for growth, or any other reaction, at any desired period after irradiation.

METHODS.

A considerable amount of work has been carried out in the last 20 years, chiefly in America and in Germany, the results of which have shown that protozoa can be kept in a constant equilibrium of growth by any appropriate means which ensures an adequate food supply and the removal of waste products. The method chosen is based upon this body of knowledge adapted to the special conditions. (Hartmann (1921 and 1928), Beers (1928), Belar (1924), Woodruff (1917 and 1925), Jollos (1916).)

It has been found that a strain of *Bodo caudatus* can be cultivated in Petri dishes at a constant number of generations per growth-period of $21\frac{1}{2}$ hours by the daily addition of a bacterial suspension in a fluid medium. The fluid of the bodo culture in the plate is removed into a tube; it is then counted and diluted to one-half the dilution of the inoculum desired for the new plate; the final dilution is made by the addition of the suspension of food bacteria. By this means

a differential dilution of the numbers of cells can be made while the food supply added remains constant. This is of great importance in the irradiation experiments where the control is growing more rapidly than the irradiated plate. The final bacterized inoculum of bodos is counted (in a Fuchs and Rosenthal counting chamber) so that the actual number can be known. The fluid used is Peters's medium (see Appendix). The bacteria are grown separately on agar slopes and are suspended in the medium, the opacity being matched with a selected tube of "Wellcome" standard opacity tubes.

The choice of the inoculum of bodos is made upon the results of a set of preliminary experiments, and an adjustment of the opacity of the bacterial suspension can be made so as to have a more or less rapid growth. Two types of plate culture have been used; in the one a layer of solid egg agar is flooded with the fluid, while in the other the egg agar is dispensed with. The purely fluid medium is the better method of culture owing to the more accurate control it affords of pH values and food-supply. The agar has been abandoned except for certain purposes for which it is well adapted.

The bodos are dependent upon the added bacterial suspension for their food and only to a very slight extent upon the growth of the bacteria during the $21\frac{1}{2}$ hours of the culture time. It should be noted that these fluid transfers do not constitute a double-culture method as the bacteria have made their growth before being placed in the bodo culture. Excellent continuous growth of the bodos is obtained in daily transfers with these bacterial suspensions when non-nutrient fluids are used, such as Peters's medium without the organic element, or even distilled water.

The plates contain 6 c.c. of fluid, and this quantity can be divided at any transfer at the time of dilution so as to afford the same inoculum for two or more plates. The counted series of transfers are made daily, and very close correspondence can be got between two sets of plates starting from one source, or from different sources, but grown by this method with similar inocula and identical food supply.

The advantage of the daily food supply being independently

grown is obvious in irradiation experiments as it affords a fresh, non-irradiated suspension on every occasion. In the longer series of continuous irradiations the possibility of a measure of variation springing up in the flora carried over at each transfer and thus effecting slight differences in the environment of the irradiated and the normal cultures, was guarded against by growing out, from time to time, the unplated bacterial complex from both sets and using these for alternate periods as the food suspension for the two plates. The food suspension is of course always shared by the two series.

A convenient rate of growth is about 4 generations in $21\frac{1}{2}$ hours. In the cultures where agar flooded with fluid medium was used this was obtained with an inoculum of about 400 bodos per cubic mm. and an opacity of the food suspension of 3 to 4 of the 'Wellcome' scale. In the cultures with the purely fluid medium this balance was got with an inoculum of about 300 bodos per cubic mm. and a food suspension of opacity 5 to 6.

The radium used in certain experiments consisted of surgical needles screened with 0.3 mm. of platinum and 0.65 mm. of lead, while in the later work the radium was contained in cells of 0.1 mm. thickness of platinum screened with 1.0 mm. of silver in a sealed applicator. Various doses were used which are indicated in the accounts of the experiments. They range from 2.88 mg. per sq. cm. to 14.77 mg. per sq. cm. The distance of the bodos from the radium was 0.5 to 0.75 cm. The radium plaque was placed in an electric incubator at 24° C. and surrounded with lead, the control plates being grown in a similar incubator and also in lead boxes. A recording thermometer was placed in each incubator and the greatest care was taken in synchronizing the temperature.

GROWTH EXPERIMENTS.

The following aspects of the growth are considered:

- (i) Continuous growth in plates transferred daily, the one set being irradiated and the control set not irradiated.
- (ii) The effect of exposure to gamma-ray irradiation during one growth-period of $21\frac{1}{2}$ hours.
- (iii) The measurement of the size of the bodos in twin plates

from one inoculum and in parallel plates, one of which is irradiated and the other not.

- (iv) Growth after irradiation. This is considered in its relation to the three sections mentioned above and not in a separate division.

Before considering these various aspects there are certain points in common that should be mentioned.

The pH values of the cultures if started at less than 7·8 always tend to move to the alkaline side during growth by 2 to 4 or more points. If the initial value is more alkaline than pH 8·0 they are inclined to move to the less alkaline side in the first few hours and then to stabilize usually at pH 7·8, while occasionally they will move to pH 8·0 or more, depending upon various conditions.

The pH values in the irradiated cultures behave exactly as in the controls; in a very small number of cases the irradiated material may be very slightly less alkaline. This is, however, exceptional, and identical pH readings (as determined by coloured indicators) at the end of the growth-period are almost invariably found.

Bodo caudatus will grow well through a very great range of pH value, from 5·7 to 9·4 or even at values of greater alkalinity, the limit of which has not been ascertained. On the acid side of pH 5·6 the cultures gradually die off.

With the doses of radium used, the highest being 14·77 mg. per sq. cm., no culture has ever been killed off. The longest period of irradiation with the highest dose was 6 days. Periods of 32 days have been used with a lesser dose of 10·38 mg. per sq. cm. or 400 mg. distributed over the whole area of 38·5 sq. cm. The screenage of 0·1 mm. of platinum and 1·0 mm. of silver makes it probable that the results recorded are effects produced by a primary emission of gamma rays from the radium source.

The factors controlled, or kept as constant as possible, in the growth of these cultures are: the food supply and the numbers dependent on it; the temperature; the pH values and salt content of the fluids used; and the free opportunity for respiratory exchange afforded by the plate type of culture. The plates

were of the same size and the amount of fluid in the cultures was always 6 c.c. All these factors have an effect upon the growth, and the irradiated cultures respond also to these conditions, but in a modified degree.

I. The Effect of continued Daily Irradiation.

In this type of experiment the start is made from a given plate of the normal strain after it has been growing in the daily transfers for some days and has been stabilized at the chosen rate of about 4 generations in the $21\frac{1}{2}$ hours' growth.

The material from the chosen plate is counted and diluted and the food suspension added, the constant quantity (6 c.c.) is placed in each of two exactly similar sterile glass plates; one is placed in the control incubator in a lead box, the other on the radium plaque also enclosed in lead. The following day the material from each is removed (at the same time every day); the two counts are made in Thoma Zeiss chambers and the two transfers are carried out, the final inocula also being counted. During the first day the two cultures are twin plates, thereafter they form two parallel series, one on radium and the other constituting the free control. Twin plates in this context always mean plates from one inoculum. The free control means a similar plate sharing the same added food suspension as the irradiated one but carrying on after the first day from a separate inoculum.

Table I gives the results of several typical experiments. The data are arranged in the form of generations per growth-period of $21\frac{1}{2}$ hours. The figures could have been calculated to a 24-hour division rate, but it seemed undesirable to introduce a hypothetical figure when one based on the actual growth-time could be used. To compare the growth on the irradiated plates and the normal the averages of 6 days' continuous growth are taken, and the irradiated growth is given as a percentage ratio of the normal. A factor certainly affecting growth is the number of bodos in the inoculum, and as the exact inoculum desired can only be obtained approximately the ratio of the average inoculum of bodos in the **O** (non-irradiated) and the **R** (irradiated) plates is used to correct the ratio figure. In the other

columns the actual averages of the generations per daily growth-period of $21\frac{1}{2}$ hours are given and are not corrected by this factor.

The generations are calculated by the formula

$$\text{Generations} = \frac{\log n(2) - \log n(1)}{\log 2}$$

where $n(1)$ is the count of the starting numbers and $n(2)$ is the count of the final numbers of bodos in the culture.

$$\text{The generation-time is} = \frac{t(2) - t(1)}{\text{generations}}$$

where $t(2)$ is the growth-time of the second count and $t(1)$ is the growth-time of the first count. In computing from the start of the culture, time (1) is of course zero.

The formula rests upon two assumptions: first, that the growth is continuous at the same rate between the two counts; and secondly, that the number of cells removed by death and disintegration is negligible. The growth is actually, as will be shown later on, not quite the same through the period, although the lag is much reduced by the food supply being given as a separately grown, bacterial food suspension, in comparison with protozoan cultures where the protozoa have to await the proliferation of bacteria before getting the food supply necessary for growth. The second assumption is discussed later and does not seem to be a factor of importance.

Table I, which gives only the data of the generations, is supplemented by Table II where the criticism of the use of the formula for generations is obviated. The figures are given in terms of the average inoculum and the multiples of this produced in the two series in the plates during the daily growth-period of $21\frac{1}{2}$ hours. This method of giving the data shows the actual performance of the cultures and permits of the estimation of the possible importance of other factors such as, for instance, the absolute number of cells exposed. Correction by the ratio of the inoculum is also made here in the ratio column but not in the column giving the multiples.

In experiments **20—R** and **21—R** the radium was in the form of surgical needles screened with 0.3 mm. of platinum in the needles themselves, and in addition the lead screen of 0.65 mm.

TABLE II
The Average Growth per Period of 21½ hours in **O** Normal and **R** Irradiated Plates. The Average is for 6 Consecutive Days.

Expt.	Dose per sq. cm.	Duration of Irradiation and Growth.	Average Inoculum in O per c.mm.	Average Inoculum in R per c.mm.	Number on Plates ex- pressed as Multiples of Inoculum.		Percentage Ratio of the Average Number on the O and R Plates, Corrected.	Remarks.
					O Plates.	R Plates.		
20— O	14.77 mg.	6 days	423.3	406.8	16.30	11.10	100 : 65.36	Agar-flooded medium.
20— O								
21— O	10.03 mg.	6 days	414.1	421.3	18.94	14.30	100 : 76.87	Agar-flooded medium.
21— R								
23— O	10.38 mg.	6 days, 1st to 6th day inclusive	313.0	306.0	17.27	11.57	100 : 65.65	Purely fluid medium. Silver plaque.
23— R								
23— O	10.38 mg.	6 days, 16th to 21st day inclusive	308.0	306.0	17.96	12.5	100 : 68.89	Purely fluid medium. Silver plaque.
23— R								
23— O	No radium	6 days, 1st to 6th day inclusive	307.6	312.8	O plates 18.76 R Str. plates 16.71		100 : 90.51	After removal from radium. Irradiation lasted 32 days.
23— R . Str.								
23— O	No radium	6 days, 7th to 12th day inclusive	323.8	301.0	18.65	16.50	100 : 82.12	After removal from radium.
23— R . Str.								
23— O	No radium	6 days, 13th to 18th day inclusive	339.0	316.6	21.23	18.38	100 : 80.7	After removal from radium.
23— R . Str.								
23— O	No radium	6 days, 93rd to 98th day inclusive	299.5	300.0	21.20	21.43	100 : 101.14	3 months after removal from radium.
23— R . Str.								

thickness was used. In these two experiments the estimated amount of radium is only approximate as no recent tests had been made of the needles. These two experiments were made with the flooded-agar medium. They can be compared with each other but not quite strictly with experiment **23**, for although the rate of multiplication is much the same the total numbers are lower in **23** (see Table II), the medium is without the agar, and the radium is contained in the silver plaque, the screenage being 0.1 mm. of platinum and 1.0 mm. of silver.

In experiment **23—R** the radium (Table I) seems more effective than in **21—R**, but the conditions are not exactly the same and one hesitates to draw the conclusion that the smaller number of cells exposed in **23** has any bearing on the result.

The ratio of growth between the normal and the irradiated plates expressed in generations is 100 to 83.79 in the first 6 days, and 100 to 86.54 in the 16th to the 21st days of a continuous series of irradiation. There were slight differences in the culture at the two periods. In **23** the pH value in the first 6 days was 7.3 to 7.4 at the start of growth and 7.8 at the end of the 21½ hours, with a food suspension of opacity 4 rising to 5. In the second set (16th to 21st day) the pH was 6.1 at the beginning of growth and 7.0 to 7.1 at the end, and the food suspension was equal to opacity 5 rising to opacity 6. It seems reasonable to conclude that the slight discrepancy has no significance and that there is no evidence that the bodos become acclimatized to the gamma-ray irradiation. This is also borne out by evidence in the next section.

Experiment **17—O** (Table I) gives the comparison in flooded-agar cultures between a normal plate series and a radium sub-transfer series, that is a series which had been exposed to irradiation (with 6.6 mg. per sq. cm. for 19 days) and was then carried on in a series of transfers in the absence of the radium. The 6-day average given here is from the 16th to the 21st day, and it will be observed that the normal growth has been resumed by the irradiated material. This also shows what close correspondence is maintained between two plates with as nearly equal inoculum as is possible and with equal food supply.

In Table II the last four items are the growth of the plates

in experiment **23** after the irradiation had been concluded. The irradiated strain had been exposed for 32 days, and the two series the normal (**O**) and the radium subtransfers (**R. Str.**) were carried on in counted transfers in the absence of the radium. After this degree of irradiation the irradiated strain is not quite able to reach the standard of growth of the normal. The discrepancy is definite but not great.

In the last division in Table II the two strains had been released from the transfer cultures and were allowed to remain for 17 days in tubes of Peters's medium unbacterized except for those taken over with the bodos. During this period growth was much reduced. They were then grown in cycle plates of flooded agar where the growth of the bacteria and the bodos takes place in true double culture at room temperature without added food supply for 68 days and 29 subcultures, and were finally both put into the daily transfer type of culture at 24° C. with the bacterial suspension as daily food supply. The two strains now grew at a rapid rate owing to the use of a more alkaline medium (starting pH 8.4 to 8.6, final pH at the end of growth at 21½ hours, 7.8) and the high food supply of opacity 6. The correspondence in numbers was extremely close and the radium effect had apparently disappeared.

The figures for this last period, calculated as generations, amount to 4.408 for the normal strain and to 4.421 for the previously irradiated strain. It is interesting to note that the rise in the performance of the normal plates, which as shown in Table I (**23—O**) were producing 6-day averages of 4.101 and 4.160 generations, is here due to the alkaline pH value and the food supply, and also to the slightly lower numbers of bodos in the inoculum. The temperature was exactly the same throughout.

The conclusions that may be drawn from these continuous irradiation experiments are: (i) that there is a definite reduction in growth in the presence of gamma-ray irradiation; (ii) that there is no evidence of acclimatization to the effect of gamma rays; (iii) that if the irradiation is prolonged and sufficiently intense there is some slight degree of reduction in the capacity for growth after removal of the radium. This is, however, not

maintained indefinitely, and though it was clearly appreciable after 13 to 18 days it had disappeared after 93 days.

II. The Effect of Exposure to Gamma-Ray Irradiation during one Growth-Period of $21\frac{1}{2}$ hours.

It is clear that the comparison of the conditions on the irradiated and the normal plates is closest upon the first day. In such a comparison the two plates are made from identical material and inoculum, and the presence of the radium is the only factor which is different in the conditions of the two cultures at the outset of the growth. The ratio of the total figures can here be used as the means of expressing the difference in growth in the two plates. (The correspondence between normal twin plates is very close; 100 to 95.9, 100 to 98.3, and 100 to 93.9 are typical ratios for the total figures on single pairs of plates.) The discrepancy in growth is usually greater on this first day than on the following days of a continuous irradiation as the differential dilution favours the growth of the irradiated series.

Taking the discrepancy in growth of such twin plates at the end of $21\frac{1}{2}$ hours, the average of four examples gives a ratio of the total figures on the normal plates to the irradiated of 100 to 57.8 (or in generations as 100 is to 81.0). The series which had been irradiated for 32 days with gamma rays from 10.38 mg. per sq. cm. was grown in the absence of radium for 14 days, and the material was then arranged in twin plates one of which was irradiated. The ratio of the total figures obtained was as 100 is to 56.7 (23—RRA), showing that there was no sign of increased resistance or acclimatization to the action of the radium.

If twin plates, one of which is irradiated, are allowed to grow only for a fraction of the growth-period of $21\frac{1}{2}$ hours, the progress of the development of the divergence between the two can be studied. Table III has been constructed from the data of a series of such plates growing under exactly similar conditions of food and temperature and as nearly identical inoculum as possible, where the growth is arrested and the counts are made at various times.

This table shows that at $5\frac{1}{2}$ hours there is already some degree

TABLE III.

Ratio of the Total Numbers of Bodos on the Normal and Irradiated Pairs of Twin Plates at different times expressed as a percentage of the Normal.

<i>Experiment.</i>	<i>5½ hours' growth.</i>	<i>6½ hours' growth.</i>	<i>8 hours' growth.</i>	<i>11 hours' growth.</i>	<i>17½ hours' growth.</i>	<i>18½ hours' growth.</i>	<i>21½ hours' growth.</i>
Twin pairs in Experiment 23. Radium 400 mg. in silver applicator = 10.38 per sq. cm.	100 : 89.0	100 : 79.27	100 : 59.26	100 : 68.9	100 : 46.6	100 : 51.25	100 : 57.82 (average of 4 pairs)

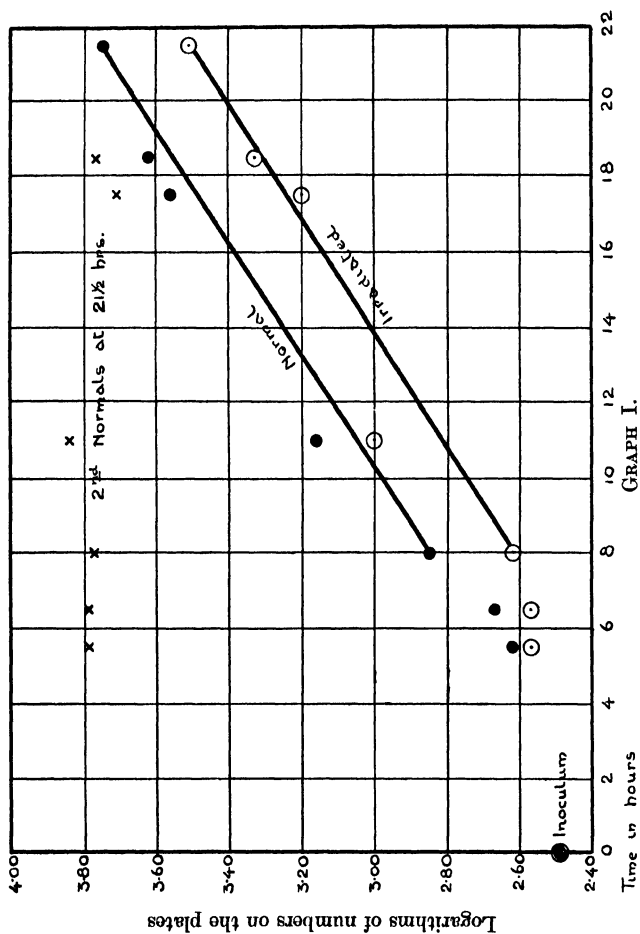
of discrepancy, though, as one would expect, it is not very great. It is represented by the ratio of the total figures on the normal and the irradiated of 100 to 89.7, while at 8 hours almost the final ratio of discrepancy is present.

When the counts of the cells on these twin plates are plotted (Graph I) as the logarithms of the numbers per c.mm., it is seen that the rate of multiplication lags in the control series till the $5\frac{1}{2}$ -hour count. It is rising steeply, however, and varies very little from the 8th hour onwards. The graph does not take into account the variations in the inoculum between the different sets of plates.

The sets of plates used in Graph I were always started in triplicate: two of these were run as controls and irradiated twins and counted at the chosen time; the third plate was allowed to grow for the usual $21\frac{1}{2}$ hours and then counted. By this means the variation between the rate of growth of different pairs can be observed and a useful check is obtained as to the importance of individual readings or irregularities. These plates are represented by the points at the top of the graph corresponding to each set.

In this graph the growth from the $5\frac{1}{2}$ -hour period to the end is, in the normal plates, almost perfectly steady except for an acceleration at 11 hours which is apparently due, at least in part though not entirely, to the higher rate of that individual day. It is a legitimate use of the formula for the number of generations to apply it as a test of the generation-time between various points in the graph. If this is done in the normal plates it is found that between the $5\frac{1}{2}$ -hour point and the 8-hour point the generation-time is 264 minutes, and between the 8-hour point and the final $21\frac{1}{2}$ hours it is 274 minutes, that is to say, there is only 10 minutes' difference and the earlier period is a little shorter than the later.

In the irradiated plates the result of this test is interesting: the generation-time between the $5\frac{1}{2}$ -hour point and the 8-hour point is 938 minutes, that is, it is extremely slow, $3\frac{1}{2}$ times as much as in the normal, but the generation-time from the 8-hour to the $21\frac{1}{2}$ -hour point is 275 minutes, that is to say, it is exactly the same as in the normal. These facts are illustrated in the



GRAPH I.

Logarithms of the numbers of bodos on sets of twin plates at different times during the growth. The starting-point is the log. of the average inoculum. The last points at 21 1/2 hours are the average of four pairs of twin plates. The points at the top of the graph are the logarithms of the numbers on the second normal twin allowed to grow for 21 1/2 hours. These points serve to show the comparative rates of growth of the different sets.

graph by the parallel positions of the points in the normal and the irradiated, though the irradiated follows a line indicating a consistently lower number of bodos. This parallel rate is not brought about in either the normal or the irradiated by a perfectly even growth, as there appears to be a true acceleration about the 11th hour, exaggerated here by the adventitious high growth on that day. In the irradiated the acceleration is more marked and there is a suggestion that the growth is progressing in waves.

Too much attention should not be paid to this wave appearance in a graph of interpolated plates, but the curve as a whole does accord with a disturbance in the rhythm of division and would agree with the data of Strangeways and Hopwood (1926), and Canti and Donaldson (1926), that the sensitive period in the cells holds back development before division, but does not prevent this process from taking place, and that those held back at one period would come into division again together at a later time.

The actual lag proper from the start at zero-time to the $5\frac{1}{2}$ -hour count in the normal is represented by a generation-time of 1,059 minutes, while in the irradiated it is 2,119 minutes.

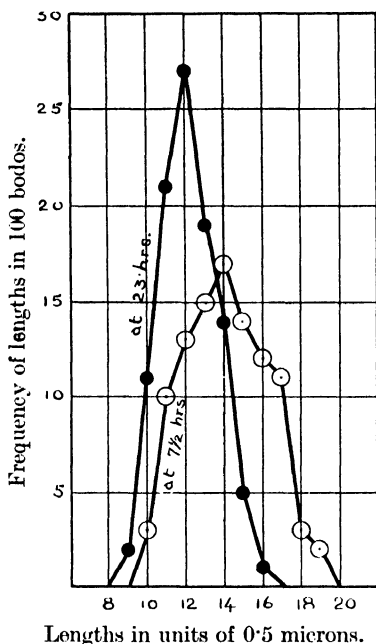
The data that this aspect of the inquiry gives is that there is a greatly enhanced lag period in the irradiated during which, however, division is still taking place, though very slowly. This is followed by an acceleration which, although it reaches an all over rate from the 8th hour onwards equal to that of the normal, starts into this phase nearly 3 hours later (or approximately one-seventh of the total growth-time of $21\frac{1}{2}$ hours) and is never able to overtake the normal in regard to numbers.

Further light is thrown on this in the next section.

III. The Measurement of the Size of the Bodos in Twin Plates and Parallel Plates, one of which is Irradiated and the other not.

The third aspect of the growth which has been considered is that suggested by the general observation that the irradiated bodos at the end of the growth-period of $21\frac{1}{2}$ hours, when the counts are made, were larger in size than the normal controls.

This impression has been tested by the measurement of the bodos in fixed and stained preparations by the methods in use for the measurement of trypanosomes and blood-corpuscles. The method proved to be quite well suited to this material,



GRAPH II.

Distribution of the lengths of the bodos in one normal plate 12—0 at $7\frac{1}{2}$ hours growth and at 23 hours, superimposed to show the difference in the position of the mean and the shape of the curves.

much more so than had at first been expected. Unfortunately this type of data was not collected for the material of Graph I. A hundred bodos from each slide were traced by the Abbé drawing apparatus at a magnification of 2,000 diameters and measured with a Zeiss mm. glass scale. The unit of measurement was 0.5 micron, i.e. 1 mm. of the scale.

The size of the bodos during the growth of the normal culture is greater in the earlier hours than later on (Graph II). There seems to be a relation between growth in size and division,

which exists at first with a bias in favour of growth and the cells are large, while, at a later period in the evolution of the plate, this relation inclines with a greater emphasis towards division and the cells gradually become smaller. The size at the end of $21\frac{1}{2}$ hours or slightly longer periods is dependent, in a constant system of cultivation and with free opportunity for respiratory exchange, mainly upon food supply and the rapidity of division; probably also to a lesser extent upon the accumulation of the waste products of metabolism. In this type of culture, where the daily dilution factor (in the normal) is relatively high (16 and upwards), a small average size at the $21\frac{1}{2}$ -hour period is, on the whole, a sign of the exhaustion of the food supply rather than an indication of the heaping up of the products of metabolism. This is shown by such data as the following.

The normal plate **12—0** running at a low rate of multiplication (3.051 generations) with a relatively low food supply gave a count of only 3,200 bodos per c.mm. with an average length of 12.17 units at 23 hours, while the normal plate **20—0** at a higher rate of multiplication (4.050 generations) gave at $21\frac{1}{2}$ hours a count of 6,625 bodos per c.mm. with an average length of 13.33 units, showing that the bodos were bigger upon the more crowded plate in response to the higher food supply and apparently indifferent to the necessarily greater amount of the products of metabolism present at the time of the count. A recent paper by Zingher, Narbutt, and Zingher (1932) gives an account of the increase in the size of *Paramoecium* in relation to food supply, and this can easily be observed also with such an organism as the ciliate *Glaucocoma*.

The measurements of the bodos from twin plates, one of which is irradiated and the other not, do reveal a larger number of big bodos in the irradiated material than in the normal. The data derived from the measurements was dealt with according to the formula used by Talliaferro (1922) for the measurements of trypanosomes.

$$M = \frac{\sum (fx)}{n}$$
 = mean where fx is the frequencies multiplied by the magnitudes and n is the total number.

$$\sigma = \sqrt{\frac{\sum x^2}{n}} \quad M^2 = \text{standard deviation}$$

where x is the magnitude of the measurements, not their deviations.

The coefficient of variation $C.V. = \frac{100 \sigma}{M}$

and expresses the standard deviation as a percentage of the mean.

The standard error of the mean $= \frac{\sigma}{\sqrt{n}}$.

The standard error of the coefficient of variation $= \frac{C.V.}{\sqrt{2n}}$

Table IV gives the mean and the coefficient of variation of the bodos upon one set of plates (twins) from which preparations were made every few hours. After 5 hours' growth one of the plates **R** was placed upon 2.88 mg. of radium per sq. cm. (surgical needles screened with platinum 0.3 mm. and 0.65 mm. of lead). This is a very small dose and the effect recorded is very slight. The plates used were of flooded-agar medium.

It will be noticed that the mean in the normal plate **12—0** falls from 14.47 units at 5 hours' growth to 12.17 at 23 hours. The coefficient of variation, which is a reflection of the variation in size brought about by the process of dividing into two, is an index of the amount of division going on. It is, however, not itself actually an index of the rate of multiplication unless some other data are given as well, such as the increase in numbers or the speed of increase in size. As it is obvious that if some condition slowed down the growth process between one division and the next, the coefficient of variation would be higher in relation to the increase in numbers than in another series where growth to the mean size was more rapid.

In Table IV the coefficient of variation in the normal plate rises from 14.43 at 5 hours to 16.49 at 10½ hours, keeps at a relatively high level (15.49 and 16.61 at 13 and 19 hours respectively) and then drops at 23 hours to 11.55. This suggests the type of increase in the division rate observed in Graph I from 5 hours onwards, and dropping as one would expect at

TABLE IV.

Table of Mean Length and Coefficient of Variation of Bodos in a Normal (12—O) and an Irradiated (12—R) Culture at different times during one (growth-period of 23 hours. The Irradiation is very slight.

	5 hours.	7½ hours.	10½ hours.	13 hours.	19 hours.	23 hours.
12—O normal twin.						
Mean length.	14.47 ± 0.20	14.10 ± 0.21	12.90 ± 0.21	13.26 ± 0.20	13.22 ± 0.21	12.17 ± 0.14
Coef. of var.	14.43 ± 1.02	15.27 ± 1.08	16.49 ± 1.16	15.49 ± 1.09	16.61 ± 1.17	11.55 ± 0.81
12—R irradiated from 5th hour on with 2.88 mg. per sq. cm. This is a small dose and effect is very slight						
	5 hr. gr. no rad.	7½ hr. 2½ rad.	10½ hr. 5½ rad.	13 hr. 8 hr. rad.	19 hr. 14 on rad.	23 hr. 18 hr. rad.
Mean length.	14.24 ± 0.19	14.12 ± 0.19	13.36 ± 0.20	13.45 ± 0.17	13.69 ± 0.17	12.98 ± 0.20
Coef. of var.	13.62 ± 0.96	13.73 ± 0.97	15.30 ± 1.08	13.06 ± 0.92	12.75 ± 0.90	15.40 ± 1.08

23 hours. In the irradiated plate only a slight discrepancy develops. The first reading, where no irradiation has been experienced, coincides extremely closely with the normal. The mean is 14.24 units, it also drops as the growth proceeds, but more slowly than in the normal, to 12.98, which is higher than in the normal, but only a little. The coefficient of variation remains stationary instead of rising at $7\frac{1}{2}$ hours' growth, after $2\frac{1}{2}$ hours' irradiation, suggesting a check in the increase of division, and while it follows the rise at $10\frac{1}{2}$ hours it is lower than the normal. It remains lower at 13 hours and 19 hours but is rising to 15.40 at 23 hours, when the normal has dropped quite definitely to the lowest figure of the series, namely 11.55. The discrepancies are slight but they are in keeping with the data of Graph I.

Graph II gives the distribution curves of the lengths of two sets of 100 bodos from plate **12—O** (the normal plate of Table IV) at $7\frac{1}{2}$ hours and at 23 hours' growth, superimposed upon one another to show the drop in size during the evolution of the culture. The graph also illustrates the difference in shape of the curves corresponding to the drop in the coefficient of variation between the two periods.

Table V gives the mean length and the coefficient of variation of the bodos in the plates in experiment **20** at $21\frac{1}{2}$ hours' growth. The top row are the normals (**20—O** to **20—O (5)**); in the second row are the irradiated series, the first irradiated plate (**20—R**) as usual being the twin of the normal, which in this case was allowed to grow for $7\frac{1}{4}$ hours before being placed upon the radium; in the third row are the daily twins of the radium series, one of which (the radium subtransfer **R. Str.**) is grown in the absence of radium. The dose of radium here was the highest used, namely, 14.77 mg. per sq. cm. The radium subtransfer set gives the daily growth of the irradiated material in fresh transfer immediately upon release from the radium.

In this experiment the normal bodos have a mean that, except upon the first day, is at the level of 12.59 to 12.22 units. The coefficient of variation varies a little but is always rather high, 15 to 20, except upon the first day when it is 14.52. In the irradiated the mean is always above 14 units except upon the

TABLE V.

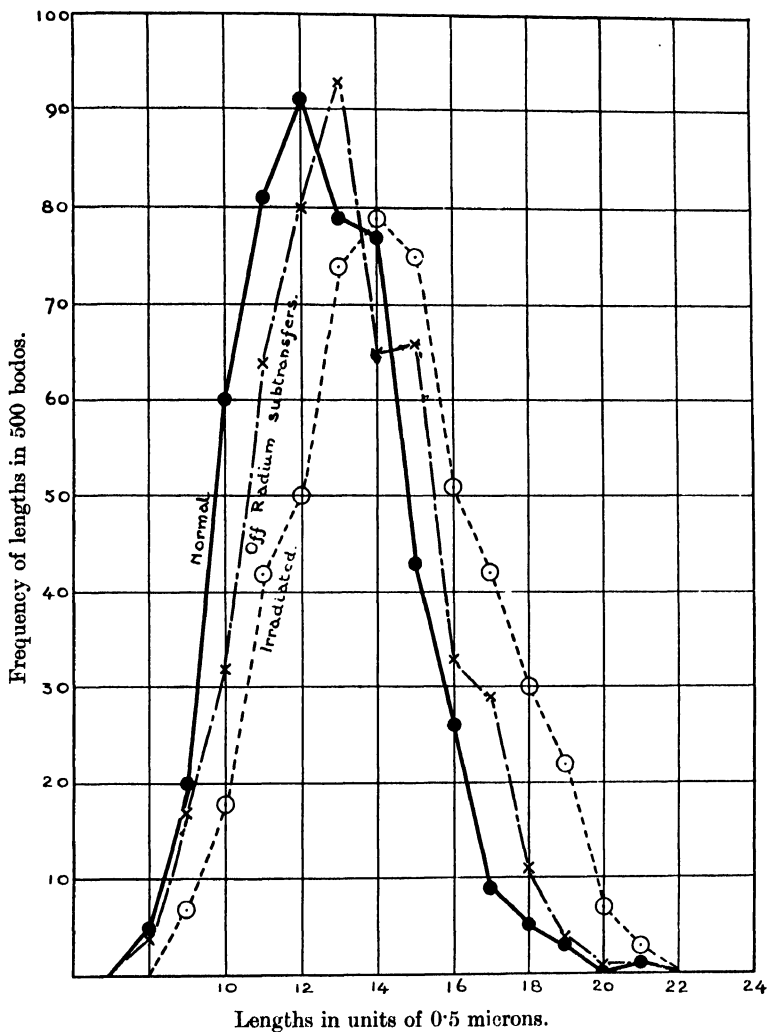
Table of Mean Length and Coefficient of Variation at 21½ hours' growth of Series 20—O (Normal) and 20—R (the Irradiated) and the First Twin of the Irradiated grown away from Radium (R. Str.).

	1st Day.	2nd Day.	3rd Day.	4th Day.	5th Day.	6th Day.
	20—O	20—O (1)	20—O (2)	20—O (3)	20—O (4)	20—O (5)
Mean length.	13.33 ± 0.19	12.59 ± 0.25	12.22 ± 0.18	12.27 ± 0.22	No preparation made	12.24 ± 0.18
Coeff. of var.	14.52 ± 1.02	20.07 ± 1.42	15.17 ± 1.07	17.96 ± 1.27	..	15.14 ± 1.07
20—O not irradiated. Free controls						
	20—R	20—R (1)	20—R (2)	20—R (3)	20—R (4)	20—R (5)
Mean length.	14.87 ± 0.25	14.37 ± 0.24	14.39 ± 0.25	13.16 ± 0.20	14.24 ± 0.25	15.10 ± 0.24
Coeff. of var.	17.25 ± 1.21	16.71 ± 1.18	17.45 ± 1.23	15.75 ± 1.11	17.73 ± 1.25	16.29 ± 1.15
20—R irradiated with 14.77 mg. per sq. cm.						
	20—R. Str. 1	20—R. (1) Str. 1	20—R. (2) Str. 1	20—R. (3) Str. 1	20—R. (4) Str. 1	
Mean length.	..	14.01 ± 0.29	12.58 ± 0.19	13.50 ± 0.21	13.21 ± 0.23	12.84 ± 0.16
Coeff. of var.	..	16.35 ± 1.16	15.65 ± 1.10	15.88 ± 1.12	17.69 ± 1.22	18.41 ± 1.30
20—R. Str. irradiated material grown away from radium						

4th day when it is 13·16; the coefficient of variation is high and fairly steady, lying between 15·75 and 17·73, indicating that division is going on actively. The discrepancy in size of the bodos in the two sets is quite definite, and it should be noted that the size of the irradiated cells in this experiment is the same at the end of 21½ hours as that of the normal ones in experiment 12 at 5 hours and at 7½ hours' growth. The relation of growth and division is producing the same mean size in the irradiated plate at 21½ hours' growth as the normal culture would at a much earlier period. The data supports the interpretation of the holding back of growth by the irradiation and the restraining of the cultures into a state which the normal has already passed through much more rapidly. There is also a contributory factor; if the bodos on, for instance, the first day are only numbering 65 per cent. of those on the control plate, the food supply must of necessity be higher per bodo in the irradiated plates, a circumstance also fostering the larger size.

In the radium subtransfers there is as a rule a slightly higher number of bodos than on the normal plates; this is an almost universally observed condition in short series of continuous irradiations. It does not seem to be a growth stimulation effect of the radium; it is apparently due to the fact that the first subtransfer away from the radium shares the low dilution of the **R** (radium) plate and therefore brings over more bacteria and also more of the growth products which within wide limits foster the growth and reduce lag in subcultures. In addition these off-radium subtransfers start with the big bodos as inoculum. The larger size in the inoculum is quite striking in the counts which are always made of the material put to grow, and seems to be a factor in increasing the rapidity of the start of division. This readiness to divide has been shown to increase the rapidity of growth in cultures of *Paramoecium* by Mitchell (1929).

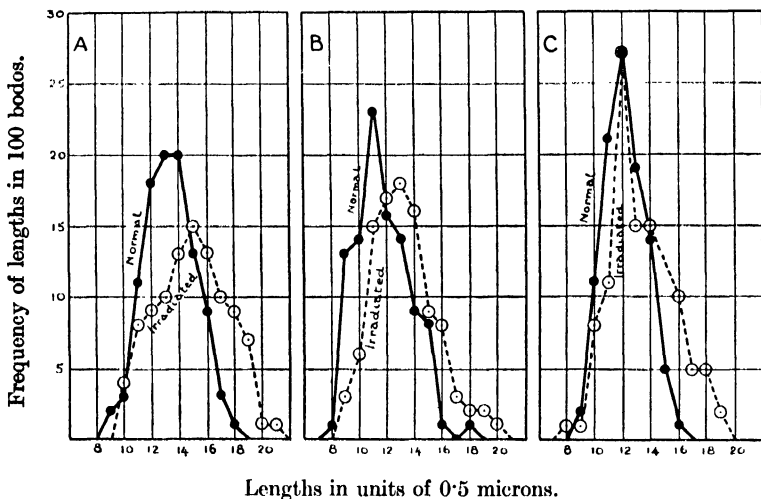
Graph III gives the distribution curves of the lengths of the bodos in experiment 20. They are composite curves of the lengths of 100 bodos from each of 5 plates of the sets comprising the normal, the irradiated, and the radium subtransfers. The graph represents the data in Table V.



GRAPH III.

Composite distribution curves of the lengths of bodos from three sets of five plates at 21½ hours' growth. The plain line represents the free controls, the dotted line represents the irradiated parallel plates, and the dot-and-dash line refers to the first off-radium subtransfers, these last being always the twins of the irradiated plates grown away from the radium.

Graph IV gives the distribution curves of the lengths of the bodos, on the normal and irradiated plates of the first day (i.e. the twin plates), of three experiments with different doses of radium. In all cases the flooded-agar medium was used. It is



GRAPH IV.

Distribution curves of the lengths of bodos on three sets of twin plates one of which is exposed to gamma-ray irradiation. *A.* 21½ hours' growth 20—O and 20—R. The R plate is irradiated for the last 14½ hours. Radium dose is 14.77 mg. per sq. cm. screened with 0.3 mm. of platinum and 0.65 mm. of lead. *B.* 21½ hours' growth 18—O and 18—R. The R plate is irradiated during the whole period. Radium dose is 6.6 mg. screened with 0.3 mm. of platinum without lead. *C.* 23 hours' growth 12—O and 12—R. The R plate is irradiated for the last 18 hours. Radium dose 2.8 mg. screened with 0.3 mm. of platinum and 0.65 of lead.

clear that the mean in **A** and **B** represents a larger size in the irradiated than in the normal. The curves in **A** and **B** in Graph IV should be compared with Graph II, when it becomes at once apparent that the curves of the effectively irradiated bodos at 21½ hours' growth resemble closely those of the normal plate in Graph II at 7½ hours. In Graph IV *C* the feebly irradiated radium plate shows a curve which differs only a little from the normal twin.

The irradiated cultures, if allowed to remain on the radium beyond the $21\frac{1}{2}$ -hour period, go on multiplying into smaller bodos but not as regularly as in the normal plates. Measurements have not been made. As time goes on the older cultures in both the normal and the irradiated are dying and encysting and changing rapidly with the accumulation of waste products and the exhaustion of the food supply, and after about 28 hours are not suitable for measurement. The irradiated cultures tend to early encystation.

DISCUSSION.

With this data of the reduction in growth, it is still open to claim that the failure in the numbers in the irradiated plates is due to the withdrawal of individuals by death. Talliaferro (1923, 1924), to whose valuable work in the elaboration of the method of measurement of trypanosomes this paper is much indebted, deduced (Talliaferro, 1922) from a high coefficient of variation and relatively low numbers in trypanosome infections that there was a process of death going on.

In the bodo material, where counts can be made at any time, it is shown (Graph I) that the rate of division is high at $21\frac{1}{2}$ hours in the irradiated cultures, but that the discrepancy in numbers is due to the check of the gamma-ray irradiation operating most effectively during the first 8 hours, whereas the normal bodos have got well under way after 5 hours. Very frequent observations of the early plates and the searching of stained preparations do not afford evidence of any serious loss through death in the plates that are irradiated. The fact that, although it is slow, division is occurring at $5\frac{1}{2}$ hours and the numbers are rising seems to preclude any significant amount of dying off. Further, it is contrary to the action of a death selection (an example of which was studied in bodo in its relation to acriflavine, Robertson (1929)) to leave no trace of its effect in the form of a subsequent acclimatization; for it is difficult to account for the relatively good growth of the survivors if there were a sensitive group on every consecutive day that was eliminated in the case of a continuous irradiation. Halberstaedter and Luntz (1929 and 1930) irradiating the green, colonial alga *Eudorina*, find that irradiation effects can be

produced by exposure before division which only become manifest at division; and exposed colonies after division are neither more nor less sensitive to reirradiation than the unexposed.

While it is clear that the radium is very effective during the early period when growth is prolonged and the interdivisional period is long even in the normal plates, it is actually less effective during the very earliest hours than slightly later, as can be seen by the fact that if twin plates are allowed to grow for 2 hours and then one is placed upon the radium, the discrepancy at $21\frac{1}{2}$ hours' growth is only very slightly reduced in comparison with twin plates where the irradiation lasted the whole growth-period; 100 to 65.5 is the (23—O (51) and 23—TR) figure obtained for the ratio of the total numbers, which is within the upper range of variation for individual twin sets where the irradiated plate was on the plaque for the whole period.

To conclude from Graph I that because the irradiated bodos are growing at the same rate as the normal after the 8th hour, they are therefore no longer restrained by the gamma-ray irradiation is erroneous. They are growing at the normal rate during that period, but they are not responding normally to the stimulus of the cultural conditions. The normal bodos are growing at that particular rate only because there are 42 per cent. more of them present absorbing the food supply and pouring out the products of metabolism. The restraint of the radium emanation upon the bodos is shown by the fact that if a plate is irradiated for the first 8 hours only and is then removed from the plaque, the growth at 18 hours (i.e. 10 hours after removal from the radium) has actually risen above the normal, and the ratio of the normal to the partially irradiated is as 100 is to 128. The irradiated bodos are here responding to the stimulus of the culture conditions, which are higher in unconsumed food and freer from the products of metabolism than the twin normal; perhaps also there is some degree of gamma-ray stimulation such as that described by Canti and Spear (1929) in tissue culture. Nasset and Kofoed (1928), irradiating *Endamoeba dysenteriae* growing in double culture with bacteria, describe a very marked stimulation effect upon the growth of this organism followed by a subsequent retardation.

It is difficult to dissociate clearly this factor of stimulation by irradiation in the cultures of bodo, as, the growth having been restrained in the presence of the radium, not only are the numbers fewer, but upon removal from the plaque the culture is in a younger state than the control as regards unconsumed food and unpolluted environment, and these have an effect upon the growth.

Some degree of evidence of a stimulation effect in these bodo cultures is found, for example, in a plate which was irradiated for $7\frac{1}{2}$ hours and then allowed to grow to the full $21\frac{1}{2}$ hours (i.e. for 14 hours after removal from the radium). It was found to be slightly below the normal, and the ratio of the total figures was as 100 is to 89.7, suggesting that the acceleration at the 18-hour period in the similar plate had been in excess of that due to the food stimulus, and was followed by a depression. The data are, however, not very clear.

If a culture irradiated for $7\frac{1}{2}$ hours is removed from the radium for $10\frac{1}{2}$ hours and then put back on to the plaque for $3\frac{1}{2}$ hours to complete the $21\frac{1}{2}$ hours of growth, this second irradiation checks the growth again so that the ratio of total numbers in the normal twin and the doubly irradiated plate is as 100 is to 74.63 instead of 100 to 89.7 where the second irradiation was not made. This does not give evidence of the relative sensitiveness of the cells at different times in the growth-period of the plate, but it does indicate that the radium is effective at the later stages also.

The literature is not discussed here, as it has been the subject of a recent review by Packard (1931).

SUMMARY.

1. The protozoon *Bodo caudatus* grown in counted cultures, which are transferred daily and supplied with a bacterial suspension (grown separately) of known opacity as food, shows a diminution in growth when grown in the presence of radium (400 mg. divided over 38.5 sq. cm. screened with platinum 0.1 mm. and 1.0 mm. of silver).

2. The ratio of the average daily number of generations in a 6-day period in the normal and the continuously irradiated

strain is as 100 is to 83.79. The ratio of the average total numbers of bodos on the plates at the end of the daily growth-period of $21\frac{1}{2}$ hours is as 100 is to 65.65.

3. There is no evidence of acclimatization to the effect of irradiation during the periods studied, namely 32 days. A test made with the irradiated strain after 14 days' growth away from radium showed a sensitiveness equal to the normal strain which had never been irradiated.

4. After irradiation for a prolonged period (32 days) with an effective dose of radium, the growth capacity of the irradiated strain is slightly impaired. This difference in growth capacity was observed for 18 days, but it had disappeared 3 months after removal from the radium.

5. The discrepancy in growth of twin plates from the same inoculum, one of which is irradiated, amounts to a ratio for the total figures as 100 is to 57.82 for the average of four pairs at the end of $21\frac{1}{2}$ hours' growth. Expressed in generations the ratio is as 100 is to 81.0.

6. The development of this discrepancy during the daily growth-period is traced, and it is shown that while the irradiation appears to be particularly effective during the first 8 hours of growth, the radium is nevertheless effective at all periods during the $21\frac{1}{2}$ hours of growth.

7. The effect of the irradiation is to delay the entry of the irradiated plate into the continuous rapid phase of multiplication by about $2\frac{1}{2}$ to 3 hours in comparison with the twin control.

8. The normal bodos are found to be larger in size in the early hours of a culture than in the later period. This change is traced by giving the mean length and the coefficient of variation in one normal plate at 6 different periods during 23 hours' growth.

9. The bodos at the end of $21\frac{1}{2}$ hours' growth are found to be larger in size in the irradiated plates than in the normal controls.

10. These data are discussed, and the mean lengths and the coefficient of variation for the normal, the irradiated, and each first plate after removal from the radium in a 6-day experiment are given.

11. It is shown that the mean length of the irradiated bodos at the end of $21\frac{1}{2}$ hours corresponds with that of the normal

bodos at a much earlier stage (5 hours and $7\frac{1}{2}$ hours). The relation of these data with that derived from the counts of the numbers of bodos in the normal and the irradiated plates is discussed.

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This work was begun in association with Mr. Bernard Williams, of St. Thomas's Hospital, and while he is not responsible for the data presented in this paper I have much pleasure in expressing my appreciation of his co-operation in various aspects of the work.

APPENDIX.

MEDIA.

The work requires a large supply of chemically clean, sterile, glass boiling-tubes, test-tubes, and pipettes, and a continuous supply of perfectly reliable media.

A. Peters's Medium.

NaCl	.	:	.	.	.	0.6 gm.
KCl	0.01 „
CaCl ₂	0.02 „
MgSO ₄	0.01 „

Glass distilled water to 1 litre.

The above solution is steamed for 15 minutes and then

Ammonium glycerophosphate (Kahlbaum) 0.6 c.c. is added. The reaction is adjusted with N/1 NaOH solution to pH 8 (or as desired).

B. Alkaline Egg Medium.

The yolk of one egg is beaten up with the whites of two. Then 6 c.c. of N/1 NaOH solution are added. When thoroughly mixed, 500 c.c. distilled water are added. This is heated in the steamer for about an hour after straining through cotton wool; it is then tubed and autoclaved.

C. Plates of Egg Agar with Peters's Medium.

The following are steamed for 15 minutes. A tube of alkaline egg medium, a flask containing 50 c.c. of Peters's medium, a flask containing 100 c.c. of 2 per cent. water agar. 7 c.c. of alkaline egg are measured into the flask of Peters's medium and 24 c.c. of this mixture are added to the melted agar. The agar is pipetted into sterile Petri dishes in the quantities desired. The final reaction of the freshly made plates is 7.4.

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The Spermatid and the Sperm of the Crab, *Paratelphusa spinigera*.

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With Plate 32.

INTRODUCTION.

THE Decapod spermatogenesis has attracted during the past a large number of workers, some of whom have given excellent descriptions of nuclear detail and centrosome, &c. To the best of my knowledge none has given an account of the Golgi apparatus and its destiny in the process of sperm-formation. A few have described mitochondria, but it is not possible to accept most of these descriptions on account of the unsuitable technique employed.

With regard to spermatogenesis generally, two of the most important contributions made during the last few years are (1) that the mitochondria (or their product) always form an envelope at least of some part of the axial filament, and (2) that the Golgi apparatus is concerned in the formation of the acrosome. But on a perusal of the literature on Decapod spermatogenesis one finds that there is no mention of the acrosome, or indeed of the Golgi apparatus; and in a few cases in which the mitochondria have been described they are not always said to lie in the tail region. Indeed the existing accounts are not only absolutely insufficient in this respect, but all writers actually describe certain bizarre structures which are unheard of in typical spermatogenesis. Consequently the Decapod sperm has been hitherto regarded as a weird cell, very different from the typical spermatozoon.

In view of the above I undertook some years ago the study of some of these so-called atypical spermatozoa, and in this first paper of the series an account of the spermatogenesis of a crab

is offered with particular reference to the transformation of the spermatid into the sperm. No attention has been paid to nuclear changes, for which reference may be made to Fasten's excellent paper (1918).

I have to thank Mr. Sukh Dayal Malik, M.Sc., for doing in ink all the diagrams published in this paper.

PREVIOUS WORK.

Fasten (1914) has reviewed the whole literature on the Decapod spermatogenesis. A reference to this paper makes it clear that practically all the workers have confined themselves to the *Macrura* and the *Anomura*. Virtually nothing had been done with the *Brachyura* till Fasten (1918) himself published a paper on the spermatogenesis of the Pacific Coast edible crab, *Cancer magister*. For a proper understanding of the somewhat complicated process of spermateleosis in *Paratelphusa*, it is essential to summarize below Fasten's account of the same process in *Cancer*.

The spermatids produced are, at first, small and their nuclei contain large masses of chromatin. The cytoplasm is homogeneous throughout and within it a rather prominent centrosome is found. Gradually these chromatin masses disappear till ultimately only one is left. This may be said to be a nucleolus-like body which resembles a karyosome.

At about this time a densely staining mass makes its appearance in the cytoplasm. This mass has been called a mitochondrial mass by Koltzoff (1906) and Binford (1913). It stains like the chromatin of the nucleus. Fasten does not consider this mass mitochondrial in nature, especially because 'in the cells under consideration no traces of mitochondria have been observed in the earlier stages of the maturation'. On the other hand, he considers it likely that it consists of chromatin which has diffused out of the nucleus.

The nucleus wanders to one pole of the spermatid, while at the opposite pole a clear vacuole makes its appearance. Sometimes two clear vacuoles may be seen, but these later flow together into a single one. At the same time the mitochondrial mass wanders in between the nucleus and the vacuole, and

ultimately fills this entire space. The centrosome increases somewhat in size and takes a position in the centre of the mitochondrial mass.

The mitochondrial mass now transforms into a ring, and the centrosome comes to occupy the centre of its inner open space. The upper portion of the nucleus also becomes located in this space. At the same time the karyosome-like body migrates upward to the middle of the upper portion of the nucleus until it comes to lie directly below the centrosome.

Simultaneously with the last-mentioned changes, a second vacuole makes its appearance in the anterior extremity of the original first, or primary vacuole. During this time the centrosome and the karyosome-like body of the nucleus unite, and elongate into a rod-like structure, the so-called central body which penetrates the inner or proximal portion of the second vacuole.

Now an opening makes its appearance in the middle of the outer or distal end of the second vesicle (vacuole). Simultaneously with this, the central body elongates still more and its outer extremity seems to hollow out into a thin tube which soon connects up with the distal opening in the secondary vesicle. As the outer end of the central body hollows out, a ring of densely staining material, the chromatin ring, makes its appearance around the outer opening of the second vesicle.

Going hand in hand with these modifications are those which take place in the mitochondria-like ring and the nucleus. These two elements fuse into a single nuclear-mitochondrial cup. At the same time the second vesicle fits more compactly into the first vesicle. Now the radial arms or rays of the spermatozoon make their appearance. They originate as outgrowths from the nuclear-mitochondrial cup, and in the finished state they are stout structures with pointed extremities. In the mature spermatozoa the rays are tightly coiled around the nuclear-mitochondrial cup.

Fasten has also described in the cytoplasm of the primary spermatocytes two densely staining chromatoid bodies, each surrounded by a clear area. During the first meiotic division the chromatoid bodies pass undivided to opposite poles of the

cell, so that each secondary spermatocyte contains a chromatoid body. During the second meiotic division it passes undivided to one pole, resulting in the formation of two classes of spermatids, one of which contains the chromatoid body, while the other is without it. The chromatoid body is soon expelled from the spermatids which contain it, thus making all the spermatids alike in structure and appearance.

Fasten worked almost exclusively with smears. Sectioned material was also used, but the smears were of the greatest service and virtually all the deductions and illustrations were made from them. Smears were fixed with Bouin's fluid while the material to be sectioned was fixed with Flemming's strong and the Meves-Duesberg modified Flemming, all of which, it may be noted, contain acetic acid.

From the above review it becomes clear that in practically the only paper available on the spermatogenesis of the crab no mention is made of the acrosome and the Golgi apparatus. The mitochondria-like mass is considered to be chromatinic in origin, and two unusual structures, the vesicles and the chromatin-ring, have been described. These are the problems which are investigated in the present paper.

MATERIAL AND METHODS.

Specimens of the crab, *Paratelphusa spinigera*, were obtained a number of times from a fresh-water stream in Kapurthala (Punjab). On their arrival they were dissected immediately. Sectioned material proved unsatisfactory for the study of spermatids. In these cells the various cell-components are so arranged that one section fails to show them all properly. Smears had, therefore, to be prepared, and they proved admirable for the study of spermateleosis. The smears on slides were fixed from one to two hours in jars containing Champy's fluid, Flemming-without-acetic, and the same diluted with an equal amount of water. After washing them in running water for some time they were mordanted and stained with 0.5 per cent. haematoxylin in the usual way. Benda's alizarin and crystal violet were also used after keeping the Champy-fixed smears first in a mixture of one part pyroligneous acid and two parts

of 1 per cent. chromic acid, and then in 3 per cent. potassium bichromate and iron-alum for varying periods. Both these methods yielded very satisfactory preparations. Bouin's fluid was used for control. Fresh sperms from the vas deferens were also studied.

OBSERVATIONS.

In the earliest spermatid the nucleus is perfectly spherical and shows a faintly staining medullary region and a darker cortical one (figs. 1-8, Pl. 32). Sometimes a small, darkly staining granule, corresponding to the nucleolus or the karyosome of Fasten, is found embedded in the nuclear reticulum (fig. 4, Pl. 32). In the cytoplasm lie the centrosome, the mitochondria, and the Golgi elements. The first is a darkly staining granule, and its position in the cell is by no means constant. The mitochondria are extremely delicate vesicles scattered throughout the cytoplasm (figs. 1-8, Pl. 32). They stain very lightly with haematoxylin or with crystal violet. Each mitochondrion shows a slightly chromophilic periphery and an absolutely chromophobic interior. They can be very aptly compared to small soap bubbles. There are a few Golgi elements lying close together usually near the nucleus. These stain much more densely than the mitochondria. Each appears in the form of a ring with a darkly staining periphery and a lightly staining interior (fig. 1, Pl. 32). Sometimes a Golgi element may appear in the form of a crescent (fig. 8, Pl. 32), or a horseshoe (fig. 7, Pl. 32), but these forms are very rare.

From now onward four changes begin to take place simultaneously in the spermatid. The first concerns the nucleus and the other three occur in the centrosome, the mitochondria, and the Golgi elements. The thin, peripheral, darkly staining area of the nucleus becomes prominent. It gradually expands inwards till the central, lightly staining area is considerably reduced (fig. 9, Pl. 32). The latter ultimately disappears altogether and the whole of the nucleus stains darkly and uniformly (figs. 11, 12, 13, 14, and 16, Pl. 32). The centrosome first becomes rod-shaped (figs. 2 and 4, Pl. 32) and then divides into two (figs. 3, 5, 6, and 8, Pl. 32). The mitochondria grow and become more resistant to acetic acid. Nevertheless, they

continue to stain but lightly. In many cases the cytoplasm is so densely packed with them that it presents the appearance of a honeycomb (figs. 1 and 5, Pl. 32). Very often very large mitochondria appear which undoubtedly are formed by the running together of smaller ones (fig. 6, Pl. 32). The Golgi rings come to be arranged more closely together and begin to stain more densely. Consequently they form one oval, or spherical, or kidney-shaped, and densely staining compact body in which, however, the individual rings may still be observed for a long time (figs. 2-8, Pl. 32). This body is destined to form the acrosome and corresponds with the 'mitochondria-like mass' of Fasten.

The process of the running together of the mitochondria continues till a large, clear vesicle is formed (figs. 9 to 13, Pl. 32) corresponding to the first or primary vesicle (or vacuole) of Fasten. A few mitochondria are left over and do not share in the formation of the mitochondrial vesicle when it first appears. There is no evidence, however, that these mitochondria are sloughed off. On the other hand, it appears that they too are gradually absorbed into the big vesicle.

Simultaneously with the completion of the mitochondrial vesicle the two centrosomes place themselves at or near its base (figs. 12 and 13, Pl. 32). Soon after one of these establishes itself on what will become the distal or posterior border of the vesicle (fig. 14, Pl. 32). It soon becomes ring-like—the so-called 'Chromatin-ring' of Fasten (figs. 15 to 18, Pl. 32). From now onward this will be called the distal centrosome. Simultaneously with this change the hitherto spherical nucleus becomes flattened out in many cases (figs. 14, 16, 17, and 22, Pl. 32).

Soon after this the nucleus becomes cup-shaped (figs. 19 and 20, Pl. 32). The nucleolus is first reduced to a faint streak (fig. 24, Pl. 32) and then disappears. The mitochondrial vesicle fits into the cavity of the cup, on the surface of which lies the acrosome (figs. 19 to 21, Pl. 32). The proximal centrosome, which was hitherto a granule, grows into a small vesicle with a thick periphery and a hollow interior. It places itself at the bottom of the mitochondrial vesicle and undoubtedly corresponds to the secondary vesicle of Fasten.

A very interesting change now comes over the acrosome. It first expands into a band (figs. 21 and 22, Pl. 32) which is rapidly converted into a ring (figs. 23, 24, and 25, Pl. 32). Even at this late stage an indication of the origin of the acrosome from the Golgi rings may be furnished by the presence of vacuoles inside it (fig. 23, Pl. 32). Gradually the acrosomal ring becomes less prominent as it begins to fuse with the margin of the nuclear cup, till in the ripe spermatozoa the two structures cannot be distinguished from each other (figs. 26, 27, and 28, Pl. 32). Thus is formed the nuclear-acrosomal cup corresponding to the nuclear-mitochondrial cup of Fasten.

Simultaneously with the above changes the nuclear cup becomes deeper and the mitochondrial vesicle fits more closely into its cavity. Consequently the margins of the cup touch the periphery of the ring-like distal centrosome, which now forms a very efficient plug keeping the vesicle well pressed into the cup (figs. 26 and 27, Pl. 32). At the same time the axial filament, corresponding to the central body of Fasten, grows from the bottom of the proximal centrosome, and, after piercing it and the mitochondrial vesicle, stops just below the distal centrosome. At its distal end the axial filament shows a very small darkly staining, transverse piece. This would, it appears, fit into the middle of the ring-like centrosome, thus supplementing the function of the latter of keeping the mitochondrial vesicle well pressed into the nuclear-acrosomal cup.

The process of spermateleosis is now completed. The spermatozoon, when looked at from the bottom (fig. 29, Pl. 32), appears as a disk. The margin of the disk stains deeply and represents the fused nucleus and the acrosome. Within this is the very faintly staining mitochondrial vesicle, in the centre of which lies the vesicular proximal centrosome, containing a darkly staining granule, the axial filament.

It has been mentioned above that the ring-like acrosome fuses with the margin of the nuclear cup so that in the ripe sperm the two structures cannot as a rule be distinguished from each other. But when fresh sperms from the vas deferens are studied in a drop of neutral-red solution two thickenings may be very often observed lying just within the margin of the nuclear cup.

These undoubtedly represent the attenuated acrosomal ring which at the point of curvature becomes visible (fig. 30, Pl. 32). Similarly in fixed preparations of the testis (e.g. Kolatchev) the two thickenings have been very commonly observed. All the other structures, namely, the nucleus, the axial filament, the proximal and the distal centrosomes, and the mitochondrial vesicle can be likewise observed in fresh sperms studied in a drop of water or normal saline.

Fasten has described radial arms or rays in the spermatozoon of *Cancer*. 'They originate as outgrowths from the nuclear-mitochondrial cup, and in the finished state they are stout structures with pointed extremities.' In the sperm of the crab, *Paratelphusa spinigera*, I have never seen rays so big as figured by Fasten. But quite often, just below the margin of the nuclear-acrosomal cup, short but prominent processes are given out which probably correspond to the rays of Fasten (fig. 26, Pl. 32). Sometimes a third process may be seen at the proximal end of the sperm (fig. 28, Pl. 32).

Lastly, two points of minor importance may be mentioned. Firstly, in some cases, the mitochondrial vesicle may contract so that an empty space appears between it and the nuclear cup (fig. 25, Pl. 32). Secondly, the proximal centrosome may become elongated (figs. 25 and 27, Pl. 32) and may even extend from the bottom of the mitochondrial vesicle right up to the distal centrosome (fig. 26, Pl. 32).

DISCUSSION.

I. Spermateleosis.

To sum up: The spermatozoon of the crab under discussion is a deep cup. The wall of the cup represents the nucleus with the ring-like acrosome fused with its margin. The mouth of the nuclear cup is very efficiently plugged by the ring-like posterior or distal centrosome. This serves to keep the mitochondrial vesicle well pressed into the nuclear cup. At the bottom of the cup in the lower region of the mitochondrial vesicle lies the vesicular proximal or anterior centrosome. From the bottom of this arises the axial filament which, piercing it and the mitochondrial vesicle, stops just below the distal centrosome.

A careful comparison of this description with that given by Fasten for *Cancer* will reveal the very interesting fact that the two tally in every detail. But, since he was using acetic acid and since the fate of the Golgi apparatus and the mitochondria in typical spermatogenesis was not yet fully understood, he failed to homologize the various sperm-components properly. This can be brought out very clearly by the study of the following table:

<i>Cancer</i> (Fasten).	<i>Paratelpusa</i> (Nath).
1. Nuclear-mitochondrial cup.	1. Nuclear-acrosomal cup.
2. Primary vacuole or vesicle.	2. Mitochondrial vesicle.
3. Chromatin ring.	3. Distal or posterior centrosome.
4. Secondary vacuole or vesicle.	4. Proximal or anterior centrosome.
5. Central body.	5. Axial filament.

Fasten never saw the mitochondria till they had run together to form a vesicle with contents sufficiently thick to resist the action of acetic acid. Not knowing how it was formed he called it the primary vacuole or vesicle. It need hardly be pointed out that the progressive increase in resistance of the mitochondria to acetic acid during spermateleosis is now a well-established fact (see Nath, 1926, for references). It will be recalled that in the spermatid of *Paratelpusa* the Golgi elements have been described as rings, or very rarely as crescents or horseshoe-shaped structures closely aggregated together. These soon give rise to a compact, deeply staining body destined to give rise to the acrosome. In spite of the acetic acid used by Fasten this body was not washed out in his preparations. He saw it and so did Koltzoff (1906) and Binford (1918). They considered it to be a mitochondrial body, although Fasten thought that it was chromatinic in origin. Similarly Fasten misinterpreted the proximal and the distal centrosome as the secondary vesicle and the chromatin-ring respectively. The central body was considered by Fasten as a structure formed by the union of the centrosome and the karyosome—a statement which cannot be accepted. In *Paratelpusa* it has been shown that the

axial filament (central body) arises from the proximal centrosome, but it has no connexion whatsoever with the intra-nuclear karyosome which gradually disappears in the course of spermateliosis.

Why are the mitochondria and the Golgi apparatus regarded as such in *Paratelphusa*? Apart from the very strong morphological evidence, into which it is quite unnecessary to enter here, there is the much stronger functional evidence. All cytologists agree that in the spermatogenesis of flagellate sperms the mitochondria form a sheath at least of some part of the axial filament and that the Golgi apparatus is concerned in the formation of the acrosome. The structures described as mitochondria and Golgi apparatus in the spermatid of *Paratelphusa* have exactly the same destiny.

This brings us to the very obvious and the most important conclusion of the present investigation—that the crab spermatozoon, in spite of its weird form, is essentially like a typical flagellate sperm. Like the latter it possesses a nucleus with which is fused the acrosome, and an axial filament, ensheathed by the mitochondrial nebenkern, extends between the proximal and the distal centrosomes.

This is not all. There are very strong reasons for believing that at the time of fertilization the fantastic appearance of the crab sperm changes into that of a typical flagellate spermatozoon. I possess many Bouin-fixed smears of the testis in which a large number of sperms have exploded, probably as the result of pressure (figs. 31 to 34, Pl. 32). In some cases (fig. 32, Pl. 32) the nucleus rounds off; in others (fig. 34, Pl. 32) it becomes irregular; and in still others (figs. 31 and 32, Pl. 32) it is converted into a shallow cup. The proximal centrosome is considerably stretched, and is represented by the thickened proximal portion of the axial filament. The latter is ensheathed by the now stretched, mitochondrial vesicle. The distal centrosome has become triangular, the distal transverse piece of the axial filament lying at the apex of the triangle. The spermatozoon is now exactly like a typical flagellate sperm with respect to all its components except that the axial filament does not here perform any locomotory function.

What causes the spermatozoon to explode at the time of fertilization I am unable to tell. It may be an increase in osmotic pressure within the mitochondrial vesicle. Whatever the cause the nucleus will be pushed into the egg as the result of the explosion.

Closely connected with this is the question of the function of the acrosome in general. The old idea that the acrosome is a perforatorium to enable the sperm head to pierce the egg membrane (Waldeyer) seems to be irreconcilable with the peculiar position and form of the acrosome in certain sperms (see Bowen, 1924), and the ring-like acrosome of the crab *Paratelphusa* fused with the posteriorly directed margin of the nuclear cup. Unaided by the rotatory movements of a flagellate axial filament the latter cannot possibly perform a boring function. Besides, in the crab, the entry of the nucleus into the egg seems to be ensured by the peculiar mechanism of explosion. What other function is to be ascribed to the acrosome it is difficult to suggest with certainty, but Bowen has suggested an interesting possibility: that the acrosome may represent the 'sperm-receptors' postulated in Lillie's theory of fertilization.

Lastly, the important point is to be noted that in *Paratelphusa spinigera* the acrosome arises directly from the Golgi elements and is not a secretory product thereof, as is the case in the genesis of many flagellate spermatozoa. In the latter (e.g. insects) a few Golgi elements (the acroblasts) are said to secrete the acrosome, and after performing this function they are sloughed off along with the rest of the Golgi material. This can, perhaps, be explained by the fact that in the crab there is no spinning out of the cytoplasm along a long axial filament, a process which invariably takes place in the spermateleosis of flagellate sperms.

II. Primary Spermatocytes and Spermatogonia.

Originally I had no intention of discussing the structure of these cells. But Fasten has described in the primary spermatocytes of *Cancer magister* two round, densely staining, chromatoid bodies each surrounded by a clear area. These bodies are distinct from the centrosome, which may often be

double. No such bodies have been discovered in the spermatocytes of *Paratelphusa*. On the other hand, there exists in the cytoplasm of these cells the typical Golgi-idiosome complex, consisting of four darkly staining Golgi granules embedded in a very distinct idiosomic area (fig. 36, Pl. 32). Two of these granules are big and invariably lie opposite each other, while the others are smaller and are likewise placed. It is possible that in the spermatocytes of *Cancer* there are only two big Golgi granules which, in spite of the acetic acid used by Fasten, escaped destruction and were described as the chromatoid bodies by him.

Similarly in the spermatogonium (fig. 37, Pl. 32) there is a Golgi-idiosome complex, but it is much smaller than that of the spermatocytes. As a rule it stains as a single, round granule, but after a careful subtraction of the stain it reveals a structure similar to that found in the spermatocyte.

In discussing the mitochondria-like mass, which is really the acrosome, Fasten considers it likely that it consists of chromatin which has diffused out of the nucleus, as 'in the cells under consideration no traces of mitochondria have been observed in the earlier stages of the maturation'. Here again Fasten erred on account of his having used acetic acid, for in the spermatocytes of *Paratelphusa* the mitochondria can be observed as extremely delicate granules existing throughout the cytoplasm. On the other hand, the technique used by me (Champy and iron-haematoxylin) has failed to bring out any mitochondrial granules in the spermatogonia (fig. 37, Pl. 32).

It will be recalled that in the spermatid of *Paratelphusa* the Golgi elements have been described as rings and the mitochondria have been compared to soap bubbles. On the other hand, both these substances exist in the form of granules in the spermatocytes. It is clear that at the commencement of spermateleosis the Golgi granules grow in size and become ring-like, thus crowding out the idiosome; and the mitochondrial granules likewise grow into vesicles with very thin walls. The growth of Golgi granules into rings has been described by Nath (1931) in the eggs of *Rana*, by Nath and Nangia (1931) in the eggs of *Ophiocephalus*, and by Bhatia and Nath (1931)

in the eggs of *Palaemon*. Similarly the mitochondrial granules of the spermatogonia grow into vesicles during the growth-period in the spermatogenesis of scorpions (Wilson, 1925) and some insects (e.g. Gatenby, 1917). Indeed the mitochondria are known to be highly plastic bodies (see Lewis and Lewis, 1914 and 1915).

SUMMARY.

The most important conclusion of this investigation is that the crab spermatozoon, in spite of its fantastic form, is with respect to its components exactly like a typical sperm. It possesses a cup-shaped nucleus with the margin of which the ring-like acrosome is fused. The mitochondria-like mass of Koltzoff, Binford, and Fasten are the fused Golgi elements destined to form the acrosome. The cavity of the cup is completely filled by the mitochondria vesicle, corresponding to the 'primary vesicle' of Fasten. The mouth of the cup is very efficiently plugged by the ring-like, distal centrosome which is identical with Fasten's 'chromatin ring'. At the bottom of the mitochondrial *nebenkern* lies the vesicular, proximal centrosome answering to the description of the 'secondary vesicle' of Fasten. Between the two centrosomes runs a thick axial filament (Fasten's central body).

Evidence has been produced that at the time of fertilization the unusual form of the sperm changes into that of a typical one.

In the primary spermatocytes of *Paratelphusa spinigera* there are no 'chromatoid bodies' as described by Fasten in similar cells of *Cancer*. On the other hand, both in the spermatocyte and the spermatogonium of *Paratelphusa*, there is the typical Golgi-idiosome complex.

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EXPLANATION OF PLATE 32.

Figs. 1–29 are drawn from smears fixed in Champy's fluid and stained with iron-haematoxylin.

Fig. 30 is drawn from fresh material stained with neutral red and fig. 35 from similar material studied in a drop of normal saline only.

Figs. 31–4 are from smears fixed in Bouin's fluid and stained with iron-haematoxylin.

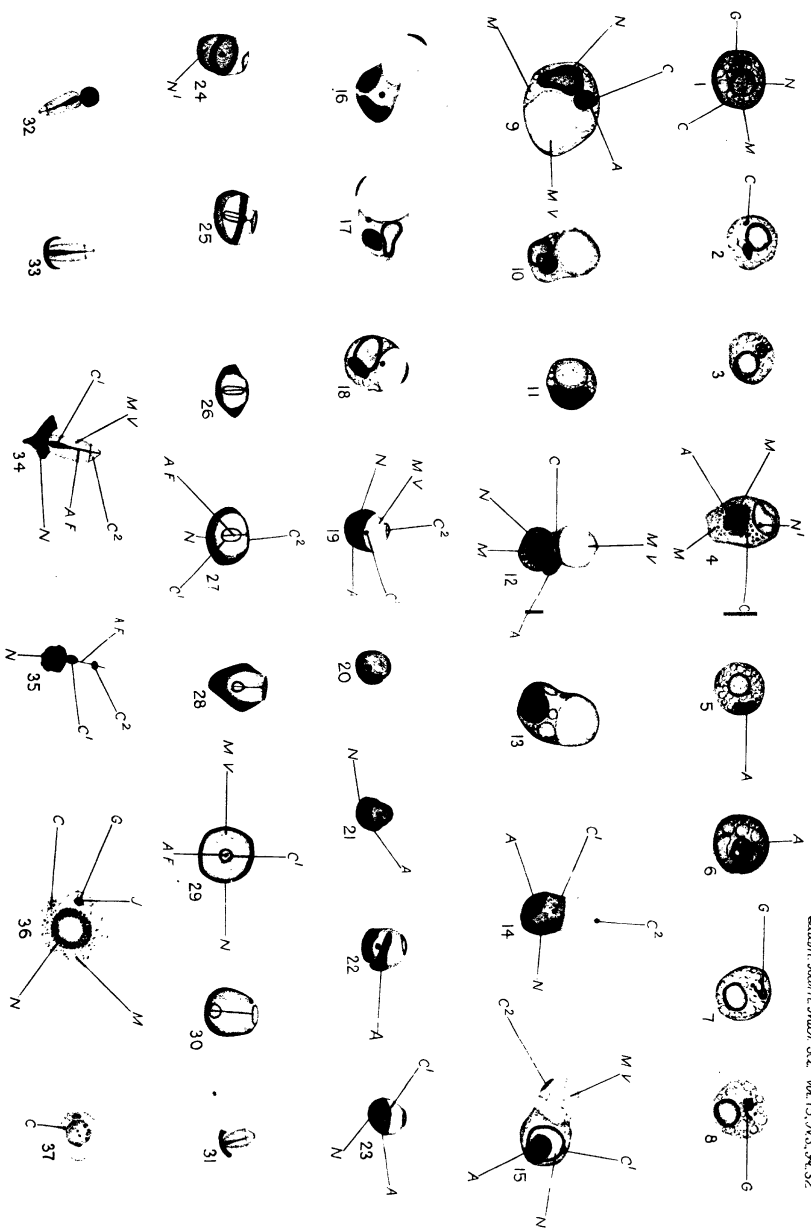
Figs. 36 and 37 are from Champy-fixed sections stained with iron-haematoxylin.

All figures except 30 and 35 are drawn with Leitz 15 × B ocular and × 95 objective at the level of the stage of the microscope, giving a magnification of approximately 1,425 times. Figs. 30 and 35 are drawn without scale.

LETTERING.

A., acrosome; *A.F.*, axial filament; *C.*, centrosome; *C*¹, proximal centrosome; *C*², distal centrosome; *G.*, Golgi element; *I.*, idiosome; *M.*, mitochondria; *N.*, nucleus; *N*¹, nucleolus; *M.V.*, mitochondrial vesicle.

Further explanation of figures will be found in the text.



The Development of the Pronephros in the Common Perch (*Perca fluviatilis* L.).

By

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With Plate 33 and 4 Text-figs.

THIS investigation into a series of *Perca fluviatilis* started some time ago.

The material consisted of a number of specimens living in an aquarium, of which some were fixed every day in Bouin's fluid. The first lot were fixed on April 7. This stage agrees with Ehrenbaum's descriptions of fish which had reached the age of twelve days. The whole embryo lay spread on the vitellus, only the head slightly lifted.

Several specimens were cut at 5 or 10 μ , most of them transversely or horizontally. They were stained with Ehrlich's (E.h.) or Heidenhain's haematoxylin (H.h.) and eosin.

While the treatment of the larvae gave no trouble at all, the young embryos, on the other hand, caused great difficulty. The large quantity of vitellus made it impossible to cut the egg at 5 μ in the ordinary way. To avoid this trouble I cut away the yolk, which can be easily done when with a small metal spatula a circular cut is made round the embryo through the outer, extra-embryonal layers surrounding the vitellus. It is then quite easy to lift the embryo from the yolk with two needles, like a lid from a box. Swaen and Brachet followed another method, for they had at their disposal living embryos, whereas the aforementioned material was fixed.

INTRODUCTION AND HISTORY.

The following is only a concise survey of the history of the development of the pronephros. In this history we may distinguish three groups of investigations or periods:

First, a morphological period, that is to say a period in which

only the development of the form was described. Rosenberg's work dates from this period (see Felix, 1897).

The second group embraces the controversy about the archinephros theory and the theory of Rückert, that is to say the question whether 'pronephros, mesonephros, and metanephros are simply successive portions of a single, elongated, ancestral, excretory organ possessing a duct and segmentally arranged, serially homologous, tubules' (Graham Kerr on the archinephros theory), or whether the mesonephric tubules are only 'eine zweite vervollkommnete Generation' of the pronephric tubules (Rückert, 1888). The first investigators of this period did not pay much attention to the state of development, so that Semon (1892) described an old stage of the *Gymnophione Ichthyophis* as a typical proof of the correctness of the theory of Rückert (1888). Some time later, however, Brauer (1902) made a particularly careful and complete investigation of the development and especially the appearance of the renal organs of another *Gymnophione Hypogeophis*. And nowadays the development of *Hypogeophis* is considered to be one of the best arguments in favour of the archinephros theory. To Kerr (1919) none of the arguments of the antagonists of this theory is 'so convincing as the very clear evidence afforded by *Hypogeophis*'.

Felix (1897) gave a good account of the development in the Teleostei; but it was to the magnificent researches of Swaen and Brachet (1899 and 1902) that we owe our knowledge of this subject. They gave an extensive description of the first appearance of the pronephros and came to the conclusion that the mode of development of pronephros and archinephric duct were principally the same. So they decided that the latter is only a large 'chambre pronéphrétique'.

Arguments in favour of the archinephros theory were brought forward by the study of Audigé (1910) on the renal organs of adult fishes.

Haller (1908) studied the development of pro- and mesonephros in Teleostei; and, using certain special considerations, he tried to form a connexion between phylogenesis and function.¹

¹ Howard and Smith (1930) tried the same for the kidneys of all vertebrates.

He concluded that pro- and meta-nephros are only differentiations of the mesonephros. In the last decennium no interest was shown in the question of the archinephros theory. Only Maschkowzeff (1926), in his study on *Acipenser*, mentioned the question.

The more precise study of the histological and micro-anatomical state of the adult renal system is characteristic of the third period.

The paper of Audigé is one of the most important studies of this period; the histological research connected with experiments led him to a consideration of the function. And we find a number of observers, partly before Audigé and partly after him, studying the histological structure. I refer to the discussion of Noll (1924), and mention the modern investigators Verne (1922), Feyel (1928), and Graham Edwards (1928, 1929).

From the above-mentioned review it follows that of late years the study of the development, especially the further development, of the pronephros of the Teleostei has been neglected, with the exception of a few observations of Verne (1922).

The following investigation is an effort to fill this gap. On one hand a description of the development of the tubule, on the other hand the development of the histological structure of the pronephros, is given.

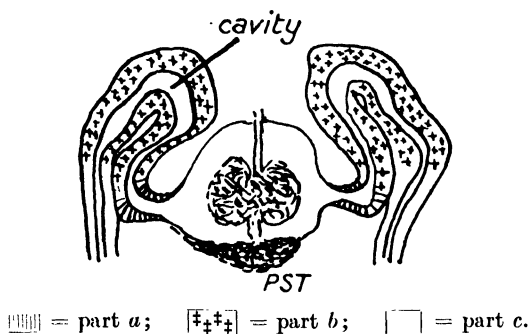
OBSERVATIONS.

For the sake of clearness I prefer to divide the following descriptions into three groups. The first two groups are confined to embryos, the third group to larvae which have left the capsules.

I. Let me begin with a brief, separate discussion on the earliest stage that was at my disposal (fixed April 8). In this embryo the alimentary canal was completely isolated from what remained of the yolk. The rudiments of the pronephros form a bulging inward of the median edge of the lateral mesoderm (fig. 1, Pl. 33). Though the lateral mesoderm is still solid, the nephrocoele is distinctly developed. The archinephric duct is folded off at full length (fig. 1 *a*, Pl. 33).

The state of development agrees with the descriptions of Swaen and Brachet (1899 and 1902).

II. Stages 1-6, fixed, April 9-14. The pronephros consists of a pronephric chamber in which the aorta forms a glomerulus. At an early stage the glomerulus fills the whole cavity of the pronephric chamber (fig. 2, Pl. 33), and the cavity does not appear until some time later. The pronephric tubule communicates with the chamber by means of a funnel. In this



TEXT-FIG. 1.

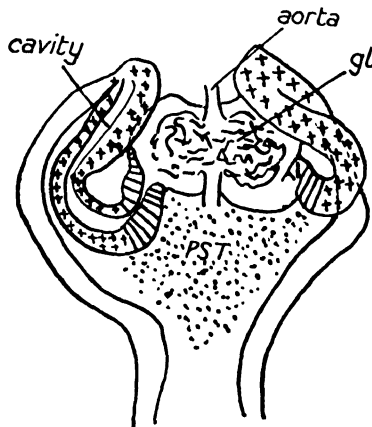
Reconstruction of the pronephros of Stage 3 (transversely cut)
(150×157).

funnel cilia are present, which are themselves not very distinct. However, we find large basal-granules (H.h.), but they begin at some distance only from the place where the epithelium of the chamber passes into the columnar-epithelium (fig. 3, Pl. 33). Audigé described this in an older stage, but I could observe it in Stage 2.

The pronephric tubule bends frontally in the earliest stages as soon as it originates from the chamber, and forms an anterior loop, after which it takes a caudal course. At a later stage it first bends caudally and forms a posterior loop. During the stay of the embryo in the capsule the differences in form are not important (cf. Text-figs. 1, 2, and 3).

The histological structure in the succeeding stages is not strikingly different. The wall of the parts of the tubule has originally a uniform structure. We notice a typical columnar-

epithelium with large nuclei. These show a chromatin network with a large nucleolus (H.h.). Sometimes there are even two nucleoli. The plasma has a somewhat bluish appearance; however it does not absorb the stain everywhere equally strongly. Frequently lighter stained streaks show themselves from the bases of the cells (fig. 4, Pl. 33). These are decidedly not fixation products; it is possible that later on they will form the spots



TEXT-FIG. 2.

Reconstruction of the pronephros of Stage 6 (horizontally cut).

through which the lymph-cells can creep. In agreement with this theory is the fact that both occur mostly in the part of the tubule stretching towards the archinephric duct.

In this duct the appearance is mainly the same as that above-mentioned, only the nucleolus is generally not very distinct.

At later stages we find differences between the parts of the tubule.

Especially in Stage 6 we notice a larger cavity in the ascending part of the tubule, while the cells are higher, which is chiefly caused by the quantity of plasma. The nuclei are situated near the base of the cells (fig. 5, Pl. 33). The descending part of the tubule forms a transition to the typical archinephric duct, which has a very small cavity.

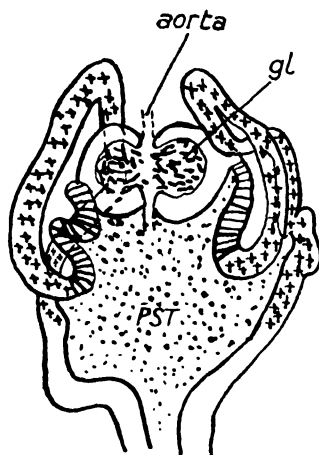
Brush-border.—In the younger stages we notice a brush-

border in the anterior loop. At later stages, however, it is more distinct (especially with E.h.).

Pseudolymphoid tissue.—Originally only situated behind the pronephric chamber, it expands at later stages and finally fills up the space between the right and left tubule.

Cardinal vein.—Ventrally to the tubules, partly situated between the parts of the anterior loop, we observe the cardinal vein (figs. 2 and 3, Pl. 33), which accompanies the archinephric duct in its further course.

III. Stages 7–13, fixed, April 15–21. These specimens are



TEXT-FIG. 3.

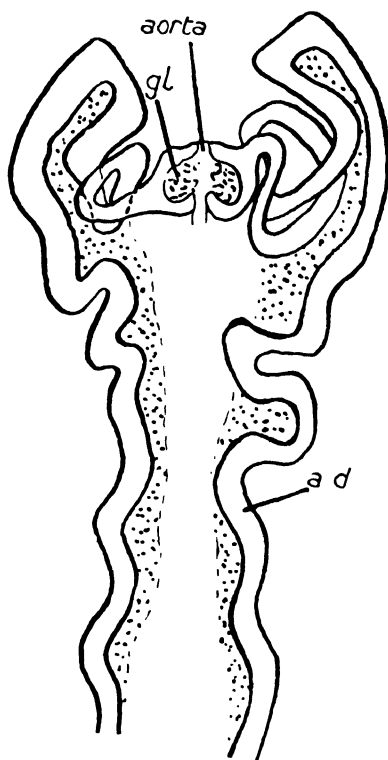
Reconstruction of the pronephros of Stage 9 (horizontally cut).

larvae, that is to say they have left the capsule. The demands of the new surroundings are demonstrated in the development of the pronephros, that is to say in the development of the form, but also in the differentiation of the histological structure.

The tubule becomes much elongated and coiled in the same way as this occurs in *Polypterus* described by Kerr (1919).

Now we may distinguish two kinds of coils: (1) the primary loops, that is to say the anterior and posterior loop; (2) the secondary coils. These vary topographically as well as in size. In later stages they may also occur in the archinephric duct (cf. Text-figs. 3 and 4). We believe that the secondary coils

are only the result of the functional demands to the tubule, that is to say a surface-increase of the latter, but that the primary loops are allied with a constitutional factor. (We must observe



TEXT-FIG. 4.

Reconstruction of the pronephros of *Lophius piscatorius* (length: 4 mm., horizontally cut).

that the latter are different in *Perca* and *Lophius*; cf. Text-figs. 2, 3, and 4.)

From an histological point of view the tubule consists of three parts: (a) the beginning of the posterior loop, which is only a continuation of the funnel; (b) the anterior loop; (c) the part that forms a transition between (b) and the archinephric duct.

(a) Audigé (1910) gave a description of this part (le collet) for a larva of *Barbus* (p. 433, &c.). I have little to add to this; besides, there is little or no difference from my descriptions of Stages 1–6, only I noticed here sometimes a slightly stained space round the nuclei.

At the posterior loop fissures penetrate the tubule between the cells and may even reach the cavity. Haller (1908) described the same for the renal tubules of the adult fishes. Often he noticed lymph-cells creeping through the fissures. Personally I cannot affirm this, but it is a matter of fact that these fissures were most distinct in older stages in which the pseudolymphoid tissue surrounded the tubule. Yet I must admit the possibility that we have to do here with artifacts.

(b) This loop is the most interesting part of the tubule.

Stage 7. In animals which have just left the capsule nothing special is to be noticed. The protoplasm shows more affinity to haematoxylin than in (a), while the situation of the nuclei is less regular (fig. 6, Pl. 33). Characteristic are the lighter stained spots that lie in the plasma. These are the rudiments of the vacuoles. Further, we notice some granules (only distinct with H.h.). They often lie against the nuclei or quite near them.

The cavity in (b) is twice or three times as big as in (a). This holds good for the ascending part; the part taking a caudal course has a less-developed cavity.

Here the cells are not so high, while we find distinct vacuoles. In one or two in the wall of the tubule I could detect a lymph-cell.

Stages 8 and 9. In older stages we find a stronger development of the lighter stained patches and vacuoles in the whole anterior loop. For this reason the protoplasm has been repelled to the base of the cells. It has a bluish appearance and is filled up with granules. These 'Körner', as Noll (1924) calls them in general, are on the one hand dispersed irregularly round the nuclei at the bases of the cells, and on the other hand they form rows stretching from tip to base in the cells.

These granules are mitochondria; that is to say they first form the rudiments of the 'réseaux protoplasmiques' described by Audigé (1910), Policard and Mawas (1906), &c. The rows of

mitochondria are the rudiments of the 'Stäbchen' of Heidenhain (1874). Benda (1903) described the same for the kidney of the embryo of a mouse (metanephros?) and the kidney (?) of a larva of *Rana*. He did not find both systems of mitochondria in one cell.

In spite of the negation of Benda (1903) I am able to confirm the observations of Audigé that the rows of mitochondria terminate in the brush-border. I must admit the possibility that we have to do with the strips of the cementing substance ('Kittleiste').

In the ascending part of the anterior loop of the tubule the protoplasm of every cell has been repelled by a vacuole and shows a triangular shape (fig. 8 *a*, Pl. 33); but in the descending part it has a more rectangular shape; here the vacuoles are less developed (fig. 8 *b*, Pl. 33).

Adjacent to the above-mentioned granules we find one or two grains lying in the vacuoles. These are of course 'grains urinaires' or granules of excretion.

Stages 11–13. These larvae do not show important differences compared with the above-mentioned stages.

Larger nuclei with a distinct nucleolus are conspicuous; the protoplasm has a granular appearance. The number of the mitochondria is diminished, but there are more granules of excretion (fig. 9, Pl. 33).

The presence of a tunica is noteworthy, for I could not detect it in younger stages.

Brush-border.—Although it is present in animals of Stages 7, 8, and 9, it is not always distinct. Whether this is the effect of the bursting of the vacuoles in the cavity of the tubule (cf. Regaud et Policard, 1902 *a* and *b*; Audigé, 1910), or an artifact, is difficult to settle.

In older stages the border is better developed and forms a bright, distinctly striated border with regularly dispersed basal-granules. So we understand that we have to do here with a ciliated border. This agrees with the observations of Nussbaum (cited by Felix, 1904).

(*c*) Conspicuous is the lack of granules and vacuoles in this part. Besides, I could not detect a brush-border round the less-

developed cavity. Practically there are no differences in the histological structure in the successive stages.

The transition between (b) and (c) is quite sudden.

Pseudolymphoid tissue.—We have little to add to the description of Stages 1–6. Now it lies even between the different parts of the tubules. Many cells are in division.

PHYSIOLOGY.

We may conclude from the preceding descriptions that part (b) of the larval pronephric tubule has an excretory function.

I have, moreover, made some observations which are not previously mentioned.

In one specimen (Stage 8, transversely cut) I could not observe a communication between pronephric chamber and tubule, neither on the left nor on the right side. The lack of this communication on the right side only I observed in an animal of Stage 9 (horizontally cut). Both animals were free swimming.

Our conclusion is that the part played by the pronephric chamber with glomerulus, which we may compare with the capsule of Bowman (cf. Haller, 1908), in the excretory function is not very important, that is to say that these observations are in favour of the theory of Bowman–Heidenhain. The aglomerular kidneys of the Lophobranch fishes support these views. Besides, Verne (1922) did not observe here a back-resorption.

Maschkowzeff (1926) admits in *Acipenser* an excretory function of the archinephric duct, while it is accompanied by the cardinal vein.¹ The histological structure in *Perca* tells in every way against this view.

DISCUSSION.

Audigé (1910) has distinguished in the mesonephric tubule of the adult Teleostei the following parts: '1, le glomérule de Malpighi avec la capsule de Bowman; 2, le collet; 3, le tube contourné; 4, le canal collecteur; 5, l'urètre.'

A comparison between his descriptions of 2, 3, and 4 and the

¹ This is not only accepted by Boveri (cf. Maschkowzeff, 1926), but also by Wheeler (1899).

preceding descriptions of the parts (*a*, *b*, and *c*) of the larval pronephric tubule shows a great similarity.

We conclude that the pronephros agrees with one mesonephric tubule of the adult fishes.

These investigations have not produced any evidence in connexion with the archinephros theory.

But as Swaen and Brachet (1899) concluded that the archinephric duct was only a large pronephric chamber, I must observe that the lack of excretory function is not in favour of this theory.

Of course it is not allowed to use physiological arguments in discussing the problem of homology; but the fact that the archinephric duct has no excretory function, in spite of the cardinal vein running along it, makes it clear that other factors, that is to say factors dependent on the constitution of the animals, are present here.

SUMMARY.

1. A description of the development of the external form of the pronephric tubule of *Perca fluviatilis* is given.

2. In two animals a communication between pronephric chamber and tubule was not found.

3. A description of the histological structure of the three parts of the pronephric tubule is given.

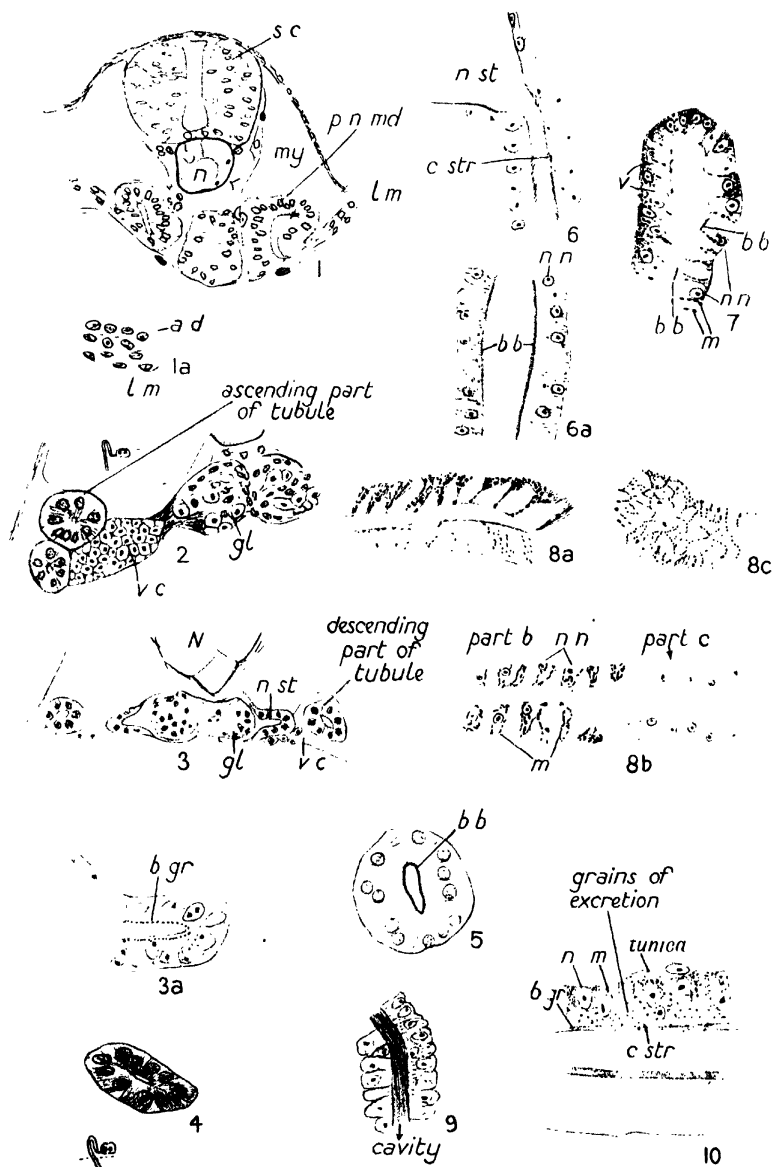
4. Three parts in the larval tubule are distinguished which agree with the parts distinguished by Audigé in the mesonephric tubule.

I wish here to acknowledge my indebtedness to the Director of our Institute, Professor J. E. W. Ihle, for the kind and helpful interest he has shown in my observations.

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EXPLANATION OF PLATE 33.

ABBREVIATIONS ON PLATE AND TEXT-FIGURES.

a.d., archinephric duct; *b.b.*, brush-border; *b.gr.*, basal-granules; *c.str.*, strips of cementing substance; *gl.*, glomerulus; *l.m.*, lateral mesoderm; *m.*, mitochondria; *my.*, myotome; *n.*, notochord; *n.n.*, nuclei; *n.st.*, nephrostome; *p.ch.*, pronephric chamber; *p.n.*, pronephros; *ps.t.*, pseudolymphoid tissue; *s.c.*, spinal cord; *v.*, vacuole; *v.c.*, cardinal vein.

Fig. 1.—Transverse section of *Perca* (fixed, April 8) through the rudiment of the pronephros (E.h.).

Fig. 1*a*.—Idem, through the archinephric duct (E.h.).

Fig. 2.—Transverse section of Stage 1 (fixed, April 9) through the pronephric chamber before the nephrostome (H.h.).

Fig. 3.—Transverse section of Stage 2 through the nephrostome (H.h.).

Fig. 3*a*.—The right nephrostome of fig. 3 (H.h.).

Fig. 4.—Transverse section through the archinephric duct (Stage 1) (H.h.).

Fig. 5.—Transverse section through the ascending part of the tubule of Stage 6 (E.h.).

Fig. 6.—Horizontal section through the nephrostome of Stage 7 (H.h.).

Fig. 6*a*.—Horizontal section through the descending part of the tubule (Stage 7) (H.h.).

Fig. 7.—Sagittal section through the posterior loop of the tubule (Stage 8) (H.h.).

Fig. 8*a*.—Horizontal section through the descending part (Stage 9) (H.h.).

Fig. 8*b*.—Horizontal section through the transition between parts *b* and *c*.

Fig. 8*c*.—Horizontal section through the network of mitochondria and strips of cementing substance (H.h.).

Fig. 9.—Horizontal section through the descending part of the tubule of Stage 13 (H.h.).

Fig. 10.—Horizontal section through the archinephric duct of Stage 9 (H.h.).

The Autonomic Nervous System of Selachians.

By

John Z. Young, B.A.

With 28 Text-figures.

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I. INTRODUCTION.

THE only complete account of the sympathetic nervous system of Selachians is that of Chevrel published in 1887. Since that date several papers have appeared dealing with special points of structure or function, such as those of Bottazzi (1902), Müller and Liljestrand (1918), and Lutz (1931), on the innervation of the viscera; of Diamare (1901) on the histology; and of

Hoffmann (1900), Müller (1920), and others, on the development. No attempt has yet been made to investigate the autonomic nervous system of these fish from the general standpoint introduced by Langley (1921) and Gaskell (1915); this the present study attempts to do. Its aim has therefore been to give an accurate account of the anatomy and histology of the system with special reference to the functions of the neurones, in order to discover whether the system conforms to the same general plan as that of mammals and whether any conclusions can be drawn either as to its phylogenetic history, or, in general, as to its nature and function. A similar study has recently been made on a Teleostean fish (Young, 1931), and the problems involved are there discussed.

II. MATERIAL AND METHODS.

The following is a list of the Selachians used in the investigation:

Order Selachii.

Group 1. Notidani.

Heptanchus cinereus.

Group 2.

Sub-order 1. Scyllioidei.

Scyllium canicula.

Scyllium catulus.

Mustelus laevis.

Mustelus vulgaris.

Carcharias glaucus.

Sub-order 2. Squaliformes.

Squalus acanthias.

Sub-order 3. Rajiformes.

Torpedo ocellata.

Torpedo marmorata.

Trygon violaceus.

The great majority of the observations were made on *Scyl-*

lium (= *Scylliorhinus*) *canicula*, the other species being used only for comparison as regards the more important points.

Most of the work was done at Naples during three visits in 1928, 1930, and 1931, when use was made of the Oxford and British Association Tables, for both of which I am very grateful, as also for the many facilities and constant assistance given to me by Professor Dohrn and all of the staff of the Station. Some material was collected at Plymouth during a short but fruitful stay at the Marine Laboratory in 1931, for which I wish to thank Dr. Allen. Much of the histological work was done in the Department of Zoology and Comparative Anatomy at Oxford, and I have to thank Professor Goodrich for much stimulating advice.

For many purposes it was desirable to anaesthetize the dog-fish, and the best method was found to be immersion in a liquid made by diluting a 10 per cent. solution of chlorotone in 95 per cent. alcohol with 99 parts of sea-water. In this they became motionless in 1-3 minutes and remained anaesthetized until revived by a stream of water passed over the gills. 2 per cent. solutions of urethane and subcutaneous injections of a 20 per cent. solution of the same were found to be effective, but less satisfactory than chlorotone. Ether anaesthesia was found to be unsatisfactory.

For study of the macroscopic anatomy of the sympathetic nervous system, use was made of the osmium tetroxide method, the advantages and limitations of which have already been discussed (Young, 1931).

For closer study of the nerves the methods of Cajal and Bielschowsky were used with success. The most satisfactory preparations were obtained by injection of the fixative after light anaesthesia, a cannula being inserted into the ventral aorta just in front of the heart. The rest of the technique was then as follows:

(1) Pieces excised and left for 24 hours in the fixative:

Chloral hydrate	5 gm.
Isotonic Selachian solution ¹	100 c.c.

¹ For the methods of preparing isotonic solutions for use with Selachians see Young, 1932.

- (2) Wash for 24 hours in changes of distilled water.
- (3) Pass slowly up the alcohol series to 95 per cent., in which leave for 24 hours.
- (4) Down the alcohol series to water and thence to 1.5–2.5 per cent. silver nitrate at 37° C. for 4 to 6 days.
- (5) Short wash in distilled water (1–5 minutes according to the size of the pieces).
- (6) Reduction in

hydroquinone or pyrogallie acid	1 gm.
neutral formol	10 c.c.
distilled water	90 c.c.

The hydroquinone reduces more violently and gives the more clear-cut pictures, but the lighter stains obtained with pyrogallol often show more detail.

- (7) Dehydrate rapidly, embed in paraffin wax and cut sections 15–30 μ thick; or alternatively, since the ganglia are very thin, whole mounts could be made in Apathy's syrup or Canada balsam, and these were found to be very useful.

This method was varied in several ways, the following being the most successful methods:

- (1) Fix in 95 per cent. alcohol plus 1 drop of NH_3 per 10 c.c.
- (2) Wash in distilled water and transfer to pyridine for 24 hours.
- (3) Wash in changes of distilled water until no smell of pyridine remains.
- (4) Proceed as above.

Or another method:

- (1) Fix for 24 hours in

ehloral hydrate	2.5 gm.
95 per cent. alcohol	40 c.c.
distilled water	40 c.c.
pyridine	20 c.c.

- (2) Proceed as in the first technique.

A number of preparations were made with the methylene blue technique, after injection of the stain, but the best preparations obtained in this way did not equal those obtained by Cajal's method. Gold chloride (Löwit's method) was found to

be useful for study of the shape of the cells, but did not stain the finer fibres.

For study of the suprarenal and interrenal systems pieces were fixed in Flemming's fluid and various formol-bichromate mixtures, of which Wiesel's fluid was found to be the most satisfactory. The best results were obtained when the solutions were made up with isotonic solution.

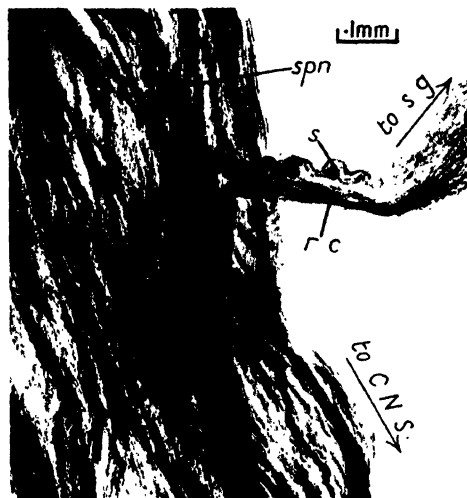
III. ANATOMY AND HISTOLOGY OF THE SYMPATHETIC SYSTEM.

1. Nature of the Rami Communicantes.

The sympathetic system of Selachians differs in its fundamental plan from that of all other vertebrates. In each segment in which it occurs there are one or more ganglia connected with the corresponding spinal nerve by a ramus communicans which consists of medullated pre-ganglionic fibres only, there are no recurrent grey rami communicantes. This condition has been revealed by study of whole mounts of rami communicantes stained with osmium tetroxide. After this treatment it can be seen that the fibres in the rami are all medullated and that at the point of junction with the spinal nerve they always turn inwards and can be followed for a considerable distance towards the central nervous system (Text-figs. 1 and 2). There are no medullated or non-medullated fibres turning outwards or running peripherally to be distributed with the spinal nerves, although such fibres if they were present would certainly be revealed by this method as they are in mammals (Müller, 1924, fig. 11) and in Teleosts (Young, 1931, fig. 3). Besides the fine medullated pre-ganglionic fibres the rami communicantes also contain sensory fibres which are very large and can be traced individually to the sense organs concerned (see p. 602).

The fact that all the motor-fibres in the rami communicantes are pre-ganglionic can be confirmed by means of preparations stained with Cajal's method, in which it can be seen that all the fibres run out to the sympathetic and none backwards from the sympathetic to the spinal nerve (Text-fig. 3).

Rami communicantes from all levels have been examined in

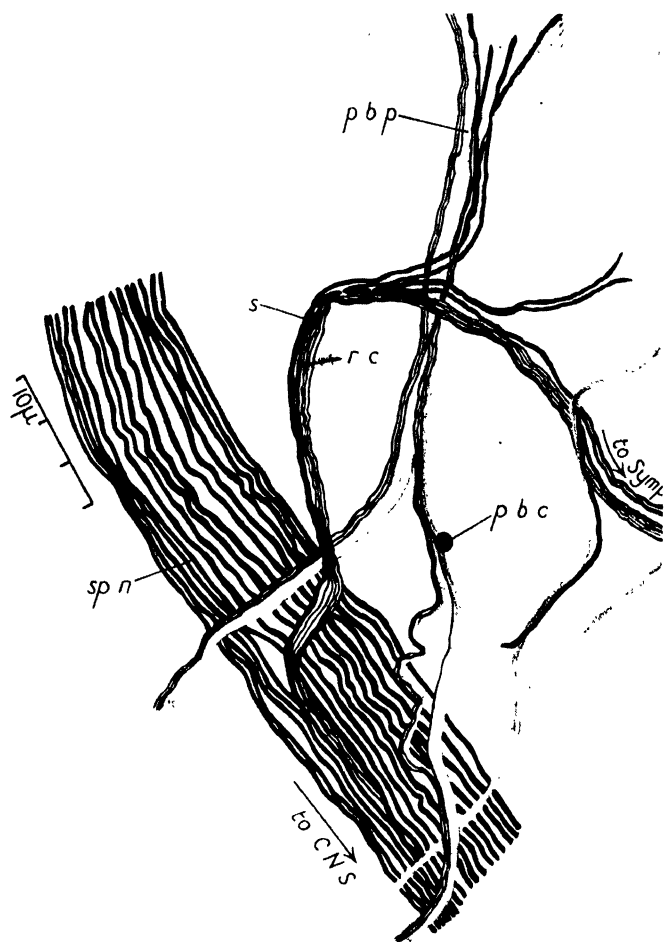


TEXT-FIG. 1.

Scyllium canicula. Origin of ramus communicans from spinal nerve. OsO_4 , whole mount. W. Watson 2/3 in., camera lucida.

EXPLANATION OF LETTERING.

a., amphicyte; *a.m.a.*, anterior mesenteric artery; *art.*, artery; *ax.*, axillary body; *b.a.*, brachial artery; *br.*, branch of non-medullated fibres; *c.a.*, coeliac artery; *c.v.*, cardinal vein; *cil.a.*, anterior ciliary nerve; *cil.p.*, posterior ciliary nerve; *cr.*, chrome-staining tissue; *d.*, dendrite; *d.a.*, dorsal aorta; *e.p.a.*, efferent pseudo-branchial artery; *f.*, nerve-fibres to chromophil cells; *g.*, gastric ganglion; *g.cil.*, ciliary ganglion; *gl.*, dendritic glomerulus; *h.*, heart; *in.*, intestine; *int.*, interrenal tissue; *k.*, kidney; *l.c.*, longitudinal connective; *n.*, nucleus of nerve-cell; *n.art.*, nerve to artery; *n.c.*, nerve-cell; *n.v.*, nerve to viscera; *not.*, notochord; *o.V + VII.*, rr. ophthalmici V + VII; *oes.*, oesophagus; *p.*, pre-ganglionic fibre; *p.b.c.*, cell of post-branchial plexus; *p.b.g.*, post-branchial ganglion; *p.b.p.*, post-branchial plexus; *ph.*, pharynx; *pr.*, r. ophthalmicus profundus; *py.*, pylorus; *r.c.*, ramus communicans; *r.s.*, sensory root of ciliary complex; *s.*, sensory fibre; *s.a.*, segmental artery; *s.g.*, sympathetic ganglion; *s.g.p.*, pre-axial sympathetic ganglion; *s.v.*, subcardinal vein; *sp.a.*, anterior splanchnic nerve; *sp.c.*, spinal cord; *sp.g.*, spinal ganglion; *sp.m.*, middle splanchnic nerve; *sp.n.*, spinal nerve; *sup.*, suprarenal tissue; *tr.*, transverse commissure; *u.*, unstained suprarenal tissue; *u.s.*, urinary sinus; *v.*, caudal vein; *v.d.*, vas deferens; *v.s.*, vago-sympathetic connexion; *w.*, whirl of fibres in sense organ; *III, IV, V, VI, VII, VIII, IX, X.*, cranial nerves; *V.m.*, rr.



TEXT-FIG. 2.

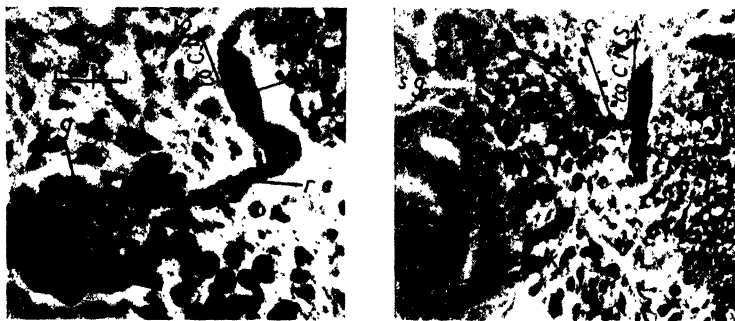
Scyllium canicula. Origin of ramus communicans from spinal nerve. OsO_4 , whole mount. Photograph, Zeiss A.

Scyllium and found to be of the same nature, and similar rami were also found in all the other species of Selachians

maxillaris+mandibularis V; *VII.h.*, r. hyomandibularis facialis;
VII.p., r. palatinus facialis.

mentioned on p. 572, so that the absence of grey rami seems to be characteristic of the group. In those vertebrates in which they occur the grey rami contain post-ganglionic fibres for the blood-vessels, melanophores, sweat glands, pilomotor muscles, and possibly also for the somatic muscles. Presumably the sympathetic has none of these functions in Selachians since, except in the case of the blood-vessels, no other tracts replacing the grey rami have been found.

The blood-vessels are innervated from the sympathetic, but



TEXT-FIG. 3.

Scyllium canicula, embryo 27 mm. Rami communicantes and sympathetic ganglia. Cajal's method. Photograph, Zeiss apo. 90, 1.3.

not via the spinal nerves. The vasomotor fibres run out from the sympathetic ganglia on either side of the segmental arteries, with which they are presumably distributed along the whole of their course. Fine branches are given off at intervals forming a complicated plexus, often containing nerve-cells, around the muscular coats of the artery (Text-fig. 4). There is also a network of fibres along the wall of the dorsal aorta, and it is probable that all the blood-vessels of the tail are supplied by this route. Thus it seems that in Selachians the vasomotor fibres accompany the arteries for long distances, whereas in mammals, according to Gilding (1932), this is never the case.

After the work of Schoenlein (1895) it was supposed that the arterial pressure of Selachians was not under nervous

control, but recent experiments have shown that stimulation of sensory nerves causes increase of blood-pressure (Huntsman, 1931) indicating that vaso-constrictor fibres are present. It is perhaps also significant that adrenaline causes constriction of the arteries (Wyman and Lutz, 1932).

In agreement with the absence of grey rami it has been found that the chromatophores of Selachians are not under nervous



TEXT-FIG. 4.

Scyllium canicula. Segmental artery with its nerves. Cajal's method, whole mount. Photograph, W. Watson 12 mm.

control. While the present work was in progress no literature could be found dealing with the question of colour change in Selachians. Since the work was finished, however, a paper has appeared by Lundstrom and Bard (1932) showing that *Mustelus* become paler in colour when brightly illuminated and that the melanophores are controlled, as in Amphibia, through the pituitary gland. v. Rijnberk (1905) considered that possibly the segmental arrangement of the spots on the body of *Scyllium* might be a result of control by segmentally distributed sympathetic nerves, but he brought no evidence to show that the melanophores were actually under nervous control. It is noteworthy that in Selachians the pattern is very stable and

there are no rapid changes in colour comparable to those seen in Teleosts, in which there is undoubted nervous control of the melanophores by post-ganglionic fibres distributed via the grey rami communicantes to the cranial and spinal nerves, cutting of which therefore causes paling over a certain area. During the present work on *Scyllium*, however, no change in the colour of the skin of the affected area was ever observed after section of the spinal, trigeminal, or facial nerves, and this leads one to suppose that there is no direct nervous control of the melanophores. The segmental arrangement of the spots, if it exists at all, may perhaps be due to the distribution of the blood-vessels.

As a further indication that the melanophores are not innervated may be cited the absence of any action upon them by adrenaline, whose action is often, though not always, parallel to that of the sympathetic system. 1 mg. of adrenaline hydrochloride (freshly made up by neutralization of the base) was injected subcutaneously into the back of a dogfish and at the same time, as a control, into a dab (*Pleuronectes limanda*). In the latter the melanophores soon contracted, first near the site of the injection and later all over the body; on the other hand, absolutely no local or diffuse colour change was observed in the dogfish. This experiment was repeated several times with *Scyllium canicula* and catulus and *Torpedo ocellata*, and was also varied by placing small pieces of the outer layers of the skin of *Scyllium* and of *Trygon violacea* in solutions of adrenaline HCl of strengths 1/5,000, 1/10,000, 1/100,000, 1/1,000,000 in sea-water. None of these pieces showed any difference from control pieces kept in sea-water, although the melanophores on the scales of a flounder (*Pleuronectes flesus*) contracted with all concentrations. Lundstrom and Bard found that *Mustelus* became somewhat paler after the injection of very large doses of adrenaline, but they reserved judgement as to whether this was a direct effect on the melanophores; probably it was a secondary effect due to vascular changes.

2. Anterior Rami Communicantes and Sympathetic Ganglia.

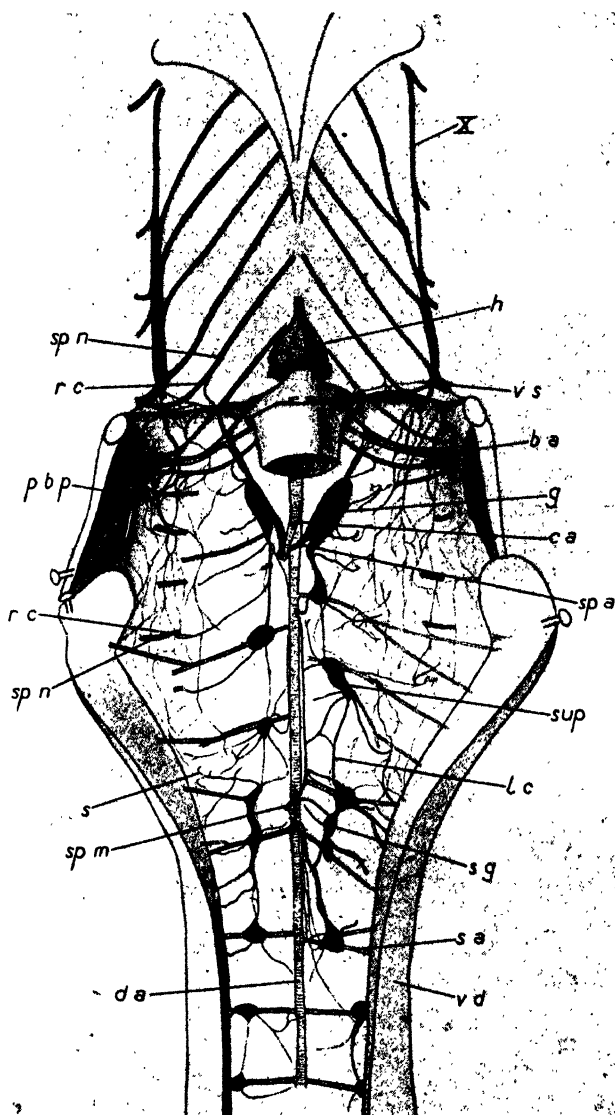
The more anterior spinal nerves do not give off rami communicantes. The position of the first ramus differs in different species and individuals; in *Scyllium canicula* it is usually connected with the third or fourth spinal nerve. The more anterior rami in all Selachians run to the large sympathetic ganglia (gastric ganglia) which are involved in the axillary bodies (Text-fig. 5). The latter are a pair of whitish bodies lying in the posterior cardinal sinus, just behind the brachial arteries, near to the point of origin of the coeliac artery. They consist of sympathetic cells and chromophil cells, partly intermixed.

The anterior rami arise from spinal nerves above the pharynx and run backwards in the loose connective tissue dorsal to the efferent branchial arteries. As they pass backwards they join to form a large bundle of fibres which enters the posterior cardinal sinus close to the brachial artery and runs into the axillary body. The gastric ganglion is not always the most anterior sympathetic ganglion in the body since there is often a small ganglion (noticed by Chevrel) on the course of the anterior rami communicantes anterior to the front end of the cardinal sinus (Text-fig. 6). From this ganglion non-medullated fibres pass to the efferent branchial arteries. Occasionally there is a small nodule of chromophil tissue close to the ganglion.

The axillary body consists of a number of fused segmental ganglia which are separate in the embryo (Hoffmann, 1900; Müller, 1920). The post-ganglionic nerves given off from the gastric ganglion are the anterior splanchnic nerves, consisting of non-medullated and lightly medullated fibres. That on the left crosses over to the right, and the two join to run with the coeliac artery to the viscera.

3. Anatomy of the Sympathetic System in the Trunk.

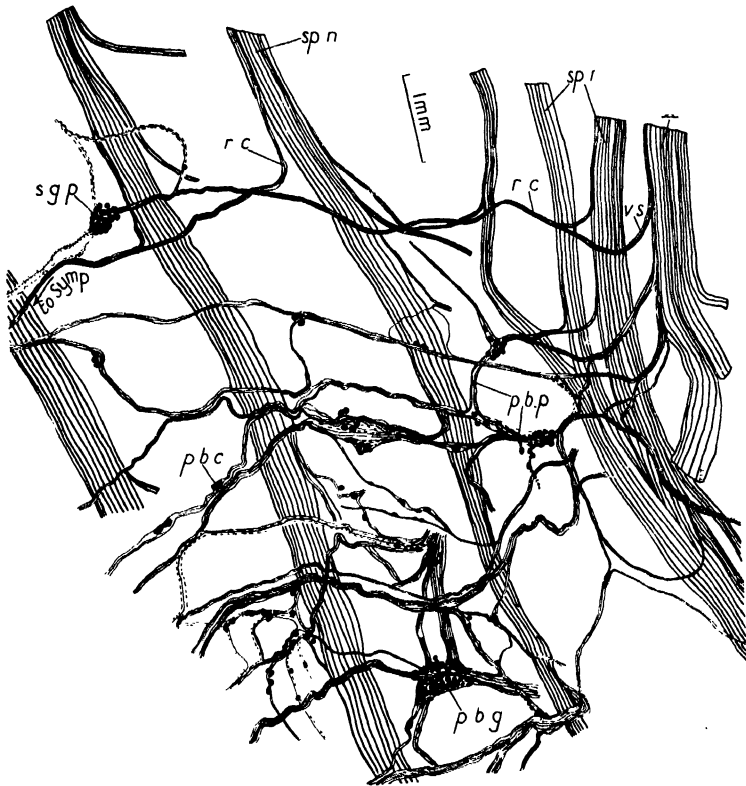
There has been considerable confusion in the interpretation of the anatomy of the sympathetic system behind the 'axillary



TEXT-FIG. 5.

Scyllium canicula ♂. Drawing of dissection of sympathetic system after treatment with OsO_4 . $\times 1/2$.

heart'. Observers (Chevrel, 1887; Bottazzi, 1902; Müller, 1920) agree that the ganglia are very small and lie in the dorsal wall of the posterior cardinal sinus, but opinions differ as to whether they are arranged segmentally, as to their relations



TEXT-FIG. 6.

Scyllium canicula ♀. Anterior rami communicantes and post-branchial plexus. OsO_4 , whole mount. Zeiss planar, camera lucida. Medullated fibres continuous lines, non-medullated fibres broken lines.

with the suprarenal bodies and as to the presence of a chain connecting them.

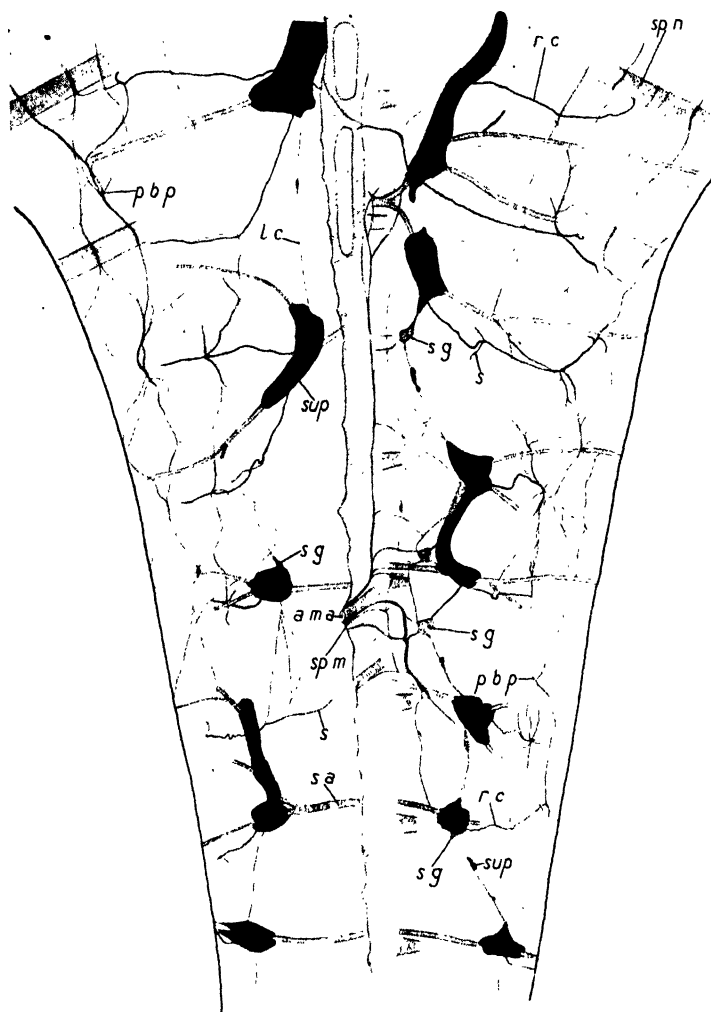
Chevrel divided these ganglia into anterior and posterior sets,

the latter distinguished by their more regular segmental arrangement and by the absence of connexions between the ganglia of adjoining segments. Bottazzi agreed with this distinction. Chevrel was emphatic in denying that the ganglia are connected to form a regular chain; he noticed that the ganglia of adjoining segments were often connected, but in other cases such connexions were absent, as also throughout the posterior interrenal series.

As will be seen from Text-figs. 5 and 7 the sympathetic ganglia are in fact very irregularly arranged. There is always at least one ganglion in every segment between the axillary body and the anus, often there are two or three of them in a single segment, connected with each other and with the ramus communicans. Anteriorly the ganglia lie immediately above the posterior cardinal sinus, posteriorly they are embedded in the dorsal part of the kidney, but the series is perfectly continuous throughout and there is no clear distinction into anterior and posterior sets. The question of the relationship of the sympathetic system to the suprarenals is best treated histologically (see p. 598); here it may be stated that in the more anterior region the ganglia often lie close to the glands (as in the case of the axillary body), but, as pointed out by Chevrel, the two may also, in any segment, be perfectly distinct (Text-figs. 5 and 7). In the kidney region the ganglia are always very close to the suprarenals.

The more anterior ganglia are very much the easier to study, since their connexions can be followed under the microscope after staining the nerves with osmium tetroxide, whereas the more posterior ganglia, being embedded in the mesonephros, can only be studied in sections. The typical arrangement of ganglia and suprarenals can be seen in Text-figs. 5 and 7. There is a ramus communicans of pre-ganglionic fibres in every segment and a number of post-ganglionic branches which are distributed to (a) the gut, (b) the suprarenals, (c) the urinogenital system, (d) the blood-vessels. The more anterior ganglia are very much larger on the left side than on the right, since most of the middle splanchnic nerves arise on the left.

Sometimes the ganglia of succeeding segments are joined by



TEXT-FIG. 7.

Scyllium canicula. Drawing of a dissection of the anterior part of the sympathetic system after treatment with OsO_4 . $\times 3$.

longitudinal connectives, but often there is no such connexion; and there is certainly no definite continuous sympathetic chain

such as that of Teleostei or Tetrapods. The connectives, when present, may contain both medullated and non-medullated fibres, but often they consist only of the latter, and the medullated fibres in the connectives usually belong to the large sense organs (p. 602), though smaller fibres, probably pre-ganglionic, may also be present. Certainly, however, there are no long paths either of pre- or post-ganglionic fibres up or down the sympathetic system. This absence of chains may be correlated with the absence of functions directly concerned with the outside world. von Frisch (1912) and Wernöe (1925) have shown that colour change in Teleosts is controlled by pre-ganglionic neurones running out in only a few segments and then passing for long distances in the sympathetic chains. It is long pathways such as these which go to make up the regular chains, obscuring what is probably a more primitive segmental arrangement, such as that which persists in Selachians.

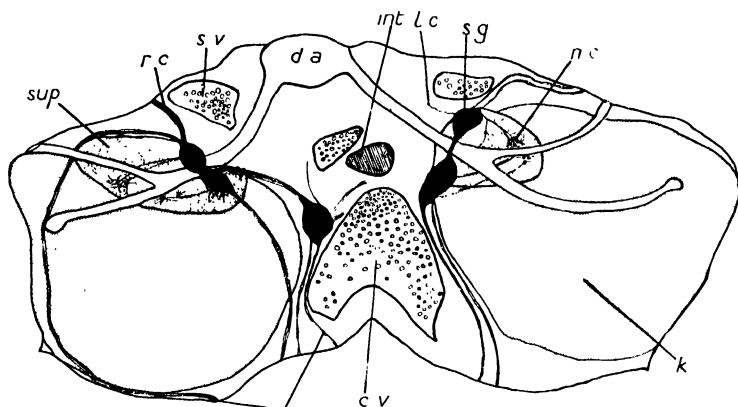
It can be seen in Text-fig. 7 that all the post-ganglionic branches are given off medially from the sympathetic and pass directly to the viscera. Their arrangement is complicated by the fact that they run irregularly backwards or forwards for short distances before passing to their destination. There is in fact a network of non-medullated nerves on either side of the mesenteries. Presumably in other vertebrates these separate post-ganglionic nerves are joined together into the sympathetic chains, although Langley (1921) was of the opinion that long post-ganglionic paths are of rare occurrence.

4. Sympathetic System and Suprarenals in the Kidney Region.

The suprarenal bodies extend along the whole length of the trunk, the hinder ones being embedded in the dorsal part of the mesonephros. In *Scyllium* and *Mustelus* the more posterior bodies have a very regular segmental arrangement, but in *Rajiformes*, where the kidneys lie farther apart, the suprarenals lie medial to them and are less regularly arranged.

The suprarenals of the kidney region differ in the two sexes. In the males of *Scyllium* they are very large and sometimes occupy a very considerable part of the volume of the kidney,

though always remaining segmental. In the females they are much smaller, and consist only of small masses of chromophil tissue around the segmental arteries, close to the origin of the latter from the dorsal aorta (Text-fig. 8). In the males, the central part only of the mass of suprarenal tissue gives the chrome reaction (Text-fig. 9), the remainder consisting of cells which seem to be of the same nature as the chromophil cells but in



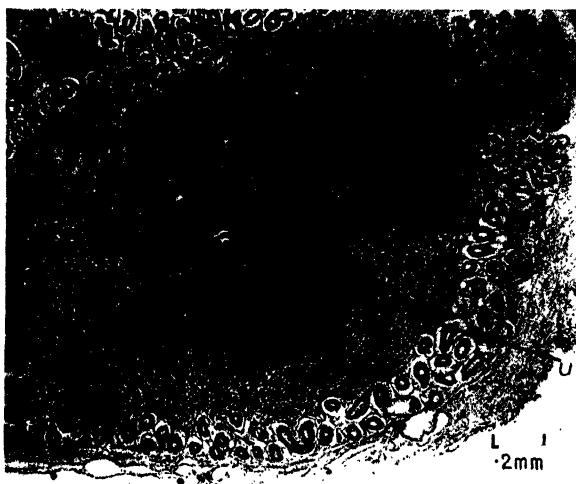
TEXT-FIG. 8.

Scyllium canicula ♀. Semi-diagrammatic representation of transverse section of kidney region. The main outlines drawn with Zeiss A lens and camera lucida from a single section, the details being filled in from the neighbouring sections.

only a few of which are there any chrome-staining granules. The total amount of adrenaline in the glands (assuming the chrome reaction to be an indicator of this) is therefore about the same in males and females, although in the former there is potentially at least twice as much adrenaline-secreting tissue as in the latter.

The sympathetic ganglia of this region always lie in close relationship with the suprarenals. The rami communicantes run through the substance of the kidney and into a ganglion which lies on the dorsal surface of the suprarenal (Text-fig. 8). From this ganglion some post-ganglionic fibres run to the suprarenal tissue (Text-fig. 18) and others, together with pre-

ganglionics, run round or through the chromophil tissue to a second ganglion which lies medial to the gland and ventral to the segmental artery. Several nerves issue from this ganglion and pass round on the medial surface of the mesonephros to run in the mesenteries to the urinogenital system or gut; often there is a third ganglion along the course of these nerves. In addition to the fibres running to the viscera there are also, contrary to the



TEXT-FIG. 9.

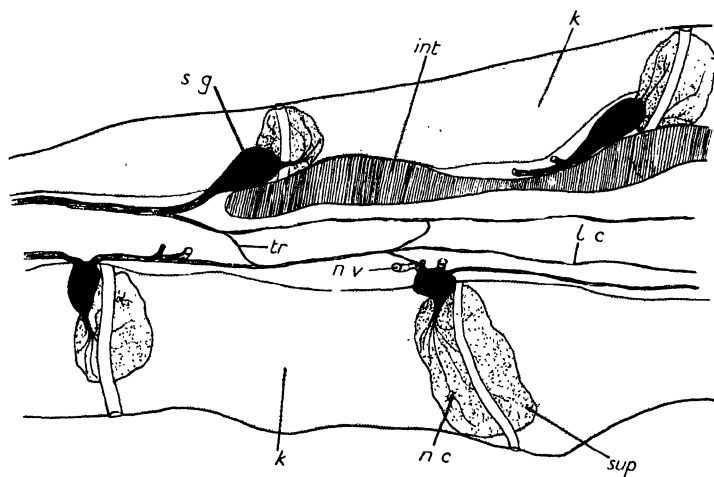
Scyllium canicula ♂. Transverse section of kidney and suprarenal. Müller's fluid with formalin, Ehrlich's haematoxylin. Photograph, Zeiss A.

opinion of Chevrel, often transverse commissures between the ganglia of the same segment and longitudinal connectives joining those of adjacent segments (Text-figs. 8 and 10). These nerves are very fine and non-medullated and can only be studied in serial sections stained with Cajal's method. They do not constitute a regular chain and may be absent in some cases. The whole system constitutes a complex arrangement giving a segmental innervation to the genital ducts, urinary sinus, intestine, and rectum.

5. Sympathetic System in the Tail.

The accounts of previous workers leave it doubtful whether the sympathetic system of Selachians extends into the tail. Thus Chevrel was unable to find any caudal ganglia in adult *Scyllium*, but Hoffmann (1900) identified them in embryo *Squalus* where apparently they became less well developed in later embryos, indicating that in the adult they may be dispersed or absent.

The observations made during the present work show that



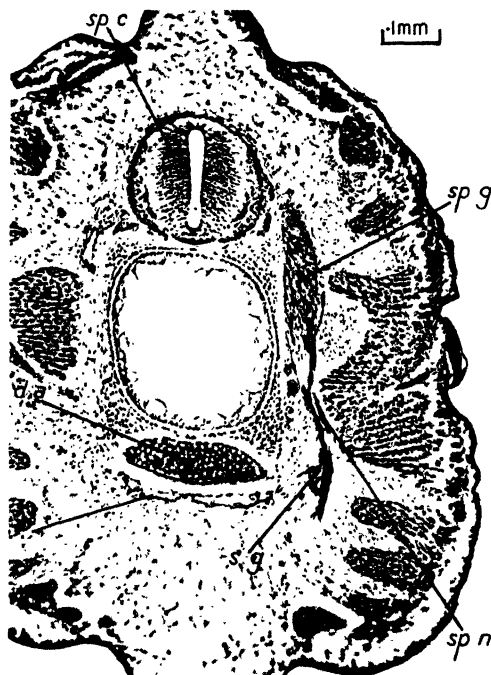
TEXT-FIG. 10.

Scyllium canicula ♀. Semi-diagrammatic representation of horizontal section of kidney region. Main outlines with Zeiss A lens and camera lucida from a single section, details from neighbouring sections.

the sympathetic ganglia and suprarenals of adult Selachians end at the hind end of the mesonephros and do not extend into the tail. This fact can best be verified in Rajiformes, where the hinder sympathetic ganglia are only partly embedded in the kidney. The last two or three ganglia are very small and the series ends where the aorta passes into the haemal canal, in which thorough search failed to reveal any further ganglia.

However, sections of embryos of *Scyllium* (25 mm.) show

that the outward migration of cells to form sympathetic ganglia does not stop at the level of the anus. Above the post-anal part of the mesonephros there are collections of sympathetic cells, connected with the spinal nerves by rami communicantes which



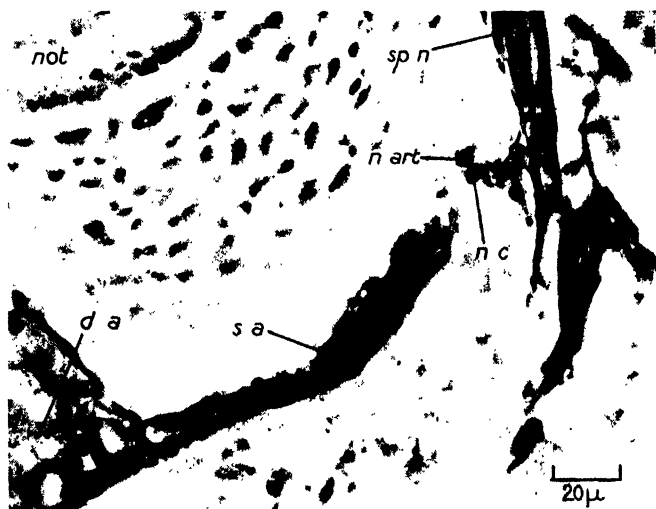
TEXT-FIG. 11.

Scyllium catulus, embryo 25 mm. Transverse section of tail.
Cajal's method. Photograph, Zeiss A.

become progressively shorter passing backwards. Behind the mesonephros these ganglia still continue for a few segments as little groups of cells between the caudal vein and the aorta, the rami eventually becoming so short that the cells lie on the spinal nerves (Text-fig. 11). In addition there are in this region twigs which pass directly from the spinal nerves to the dorsal branches of the segmental arteries (Text-fig. 12). These twigs come off just below the spinal ganglion and are therefore dorsal

to the true sympathetic ganglia. Sometimes (as in Text-fig. 12), there is a group of cells, presumably motor neurones, where the vasomotor fibres come off from the spinal nerve.

In adult *Scyllium* the post-anal sympathetic ganglia seem to disappear completely and preparations of the caudal spinal



TEXT-FIG. 12.

Scyllium catulus, embryo 25 mm. Transverse section of tail, showing nerves to artery. Cajal's method. Photograph, Zeiss apo. 90, 1.3.

nerves stained with osmium tetroxide or Cajal's method failed to reveal any cells or bundles of non-medullated fibres. It is possible that the fibres running directly from the spinal nerves to the blood-vessels still persist, the motor cells lying in the walls of the arteries; it should be possible to discover experimentally whether such fibres are present.

In the tails of two late embryo *Torpedo ocellata* (20 mm.), stained with Cajal's method, there were seen to be occasional nerve-cells scattered between the caudal vein and the aorta and connected with the spinal nerves by very fine rami. Such a cell is seen in Text-fig. 13, but it will be noticed that the

fibre attached to it runs not towards the blood-vessels, as would be expected if the cells were post-ganglionic, but centripetally towards the spinal nerve. I have not sufficient preparations to tell whether this is the usual condition of these cells. The cells are most numerous in the segments immediately behind the anus and decrease in number passing backwards. Possibly they persist in the adult as vasomotor fibres.

Thus it seems that in Selachians there are no autonomic fibres



TEXT-FIG. 13.

Torpedo ocellata. Embryo 20 mm. Transverse section of tail.
Cajal's method. Photograph, Zeiss apo. 90, 1-3.

in the tail with the possible exception of a few connected with the blood-vessels. This is as would be expected in view of the nature and functions of the sympathetic system in these fish. It has been shown above that there are no grey rami communicantes, and that the post-ganglionic fibres all pass directly to the viscera or blood-vessels. The sympathetic in the tail of other vertebrates is presumably concerned with the innervation of the skin and its derivatives, possibly also of the somatic muscles, and since the sympathetic has none of these functions in Selachians it would not be expected to extend into the tail.

6. Autonomic Fibres in Spinal Dorsal Roots.

It has sometimes been suggested that in mammals parasympathetic (especially vasodilator) fibres run out in the spinal dorsal roots, and Kurè and his collaborators have recently brought evidence purporting to show that such fibres are present in large numbers and include not only vasodilators but also motor-fibres to the gut, hair muscles, &c. It would be interesting to discover whether such fibres are present in Selachians, but the question can only be satisfactorily settled by experiment and was not fully dealt with during the present research. Sections of the dorsal root ganglia and spinal nerves failed to reveal any motor-cells, so that if such are present they must lie at the periphery. No movements of the viscera were noticed after stimulation of spinal dorsal roots by Müller and Liljestrand (1918) or in the course of the present work, so that if any visceromotor fibres are present in the dorsal roots of Selachians they must run to the blood-vessels.

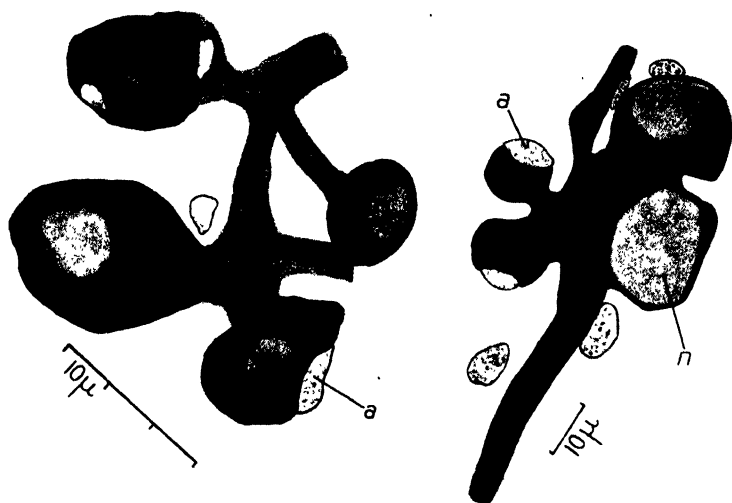
7. Cytology of the Sympathetic Ganglia.

The finer structure of the neurones of the sympathetic system of Selachians has never been fully investigated. This group was omitted from the comprehensive survey of Huber (1900) who did, however, describe the sympathetic cells of Teleosts. Diamare (1901) reported the presence of multinuclear cells in Selachians, and Müller (1920) gave an excellent description of the structure of the developing neurones. The sympathetic ganglia of mammals have been very thoroughly studied, recent accounts being those of Castro (1930 and 1932).

It is characteristic of the sympathetic cells of Selachians that they are not all concentrated into compact ganglia, many being scattered singly or in groups along the course of the nerves. In many of these groups processes of one cell make contact with other cells giving a mechanism for the spreading of impulses. Although there is no definite evidence for the existence of visceromotor chains with more than two links, yet it seems not improbable that some of these cells are intercalary neurones.

The smaller sympathetic cells have a single nucleus, but the

majority have two and some have three, four, five, or even six nuclei, and these multinucleate cells often assume curious shapes (Text-fig. 14). In the smaller ganglia the cells are often unipolar, with the cell-body at the periphery of the ganglion and the prolongation directed inwards. This prolongation then divides into a number of branches which become lost in the



TEXT-FIG. 14.

Scyllium canicula. Cells from post-branchial plexus. Formic acid, gold chloride. Zeiss apo. 90, 1.3, camera lucida.

maze of fibres (Text-fig. 15). In other cases there may be two, three, or many processes, each dividing several times. There is no means of deciding whether the conduction in these long fibres is towards or away from the cell-body, probably most of them conduct away, though their shorter branches may serve to pick up impulses from other neurones. However, there are other processes which are undoubted dendrites and these may be either extracapsular or subcapsular. The extracapsular dendrites are in the form either of short lateral branches or whirls or of many-branched, shrub-like structures which interlace with the finer branches of other cells to form 'dendritic

glomeruli' (Text-fig. 16). Often the processes of two or more cells are involved in a single glomerulus, which does not necessarily mean that the neurones concerned are intercalaries, such that stimuli pass from one to the other of them, but rather that in such whirls several cells pick up impulses from the terminations of a single pre-ganglionic axon. Similar conditions have been observed in mammals (Cajal, Castro), and it is well known



TEXT-FIG. 15.

Scyllium canicula ♀. Sympathetic ganglion in kidney region.
Cajal's method. Photograph, W. Watson 1/6 in.

that there are more post-ganglionic fibres leaving the sympathetic ganglia than pre-ganglionic fibres entering. Billingsley and Ranson (1918) calculated the ratio to be 1-32 for the superior cervical ganglion of the cat. In Selachians also there are certainly more post-ganglionic than pre-ganglionic fibres.

The finer details of the dendritic glomeruli are very difficult to make out. In each there are many amphyocytes and whirls of very fine branching fibres, which are so complex that it is difficult to be certain how they terminate. Many of them bear swellings ('boutons de passage') and possibly there are also

some terminal boutons but probably a good many of the fibres run through the whirl and out again, giving or receiving an impulse by contact without actually terminating in a blind



TEXT-FIG. 16.

Scyllium canicula. Cells from gastric ganglion. Cajal's method. Zeiss apo. 90, 1.3. Main outlines with camera lucida from a single section, details from neighbouring sections.

ending. Similar cases of synapses without terminations were seen in the post-branchial plexus (p. 600).

The subcapsular dendrites consist either (a) of whirls of fine fibres which wrap round the cell-body (Text-fig. 26) and make contact with other whirls which are the branches of pre-ganglionic fibres, or (b) of shorter processes coming off all round the

cell. These latter end as flattened plates, in which a fibrillar structure can often be made out, lying close to the amphicytes (Text-fig. 17). Other similar plates are seen attached to fibres



TEXT-FIG. 17.

Scyllium canicula. Small cell from gastric ganglion. Cajal's method. Zeiss apo. 90, 1.3, camera lucida.

which are not in connexion with the cell and are probably the endings of in-coming fibres. Similar plates were seen by Castro in the sympathetic ganglia of mammals, and he is of the opinion that every synapse is a complex structure involving boutons, dendrites, and amphicytes. However, there is no doubt that in many cases fibres run round a cell and pass on without actually terminating on that cell (see p. 600), and in these cases the fibre must transmit or receive whatever change constitutes

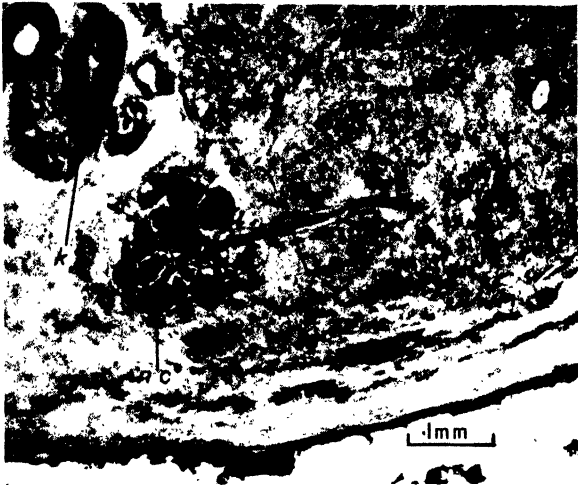
the impulse by contact, without forming a blind ending, and without the intermediation of amphicytes. Such contact is, of course, different from continuity of substance between neurones for which I have found no evidence. However, Tiegs (1927) and Stöhr have come to the conclusion that there are no discontinuous synapses in the ganglia but that 'das gesamte sympathische System ein geschlossenes Netz darstellt' (Stöhr, 1928, p. 293). This is certainly the first impression which strikes one on account of the amazing complexity of the fibres, but closer study reveals the existence of surfaces of contact taking the various forms described above, and all tending to allow of very great diffusion of impulses.

8. Relation of the Sympathetic Cells to the Suprarenal Tissue.

In many cases the sympathetic ganglia lie close to, or actually inside, the masses of suprarenal tissue. The relationship is more than one of anatomical contiguity since many large bundles of nerve-fibres pass from the ganglia to ramify among the chromophil cell (Text-fig. 18), and nerve-cells are found scattered, either singly or in groups of two or three, among the chromophil cells. The suprarenal tissue is penetrated by a very complex network of non-medullated nerve-fibres; in fact these glands are more richly innervated than any other of the viscera studied. The finer nerve-fibres wind in and out among the cells, bearing small swellings at intervals along their course and finally ending in small knobs (Text-fig. 19). The exact relationship of the finest branches with the chromophil cells is very difficult to determine; probably they make contact with the outer surface of the cells without actually penetrating into the cytoplasm.

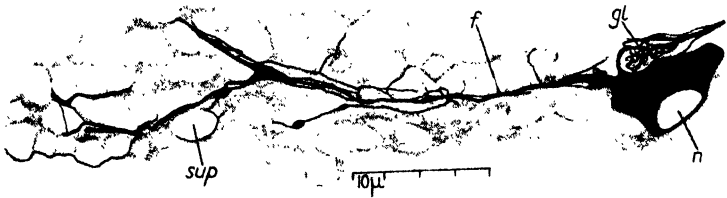
It was found possible in some cases to follow the motor-fibres from their origin in sympathetic cells to their termination around suprarenal cells (Text-fig. 19). Now Elliott (1913) was unable to discover any synapse on the path between the central nervous system and the medullary tissue of the adrenal (cat), and hence concluded that the chromophil cells (which are known to arise in ontogeny from the sympathetic anlagen) are

themselves the post-ganglionic cells, and therefore the connector fibres innervate them directly. This is certainly not so



TEXT-FIG. 18.

Scyllium canicula ♀. Transverse section of kidney region, showing sympathetic ganglia and suprarenal tissue. Cajal's method. Photograph, W. Watson 12 mm.



TEXT-FIG. 19.

Scyllium canicula. Nerve-cell from boundary between gastric ganglion and chromophil tissue in axillary body. Cajal's method. Zeiss apo. 90, 1.3, camera lucida.

in Selachians where there can be seen to be a synapse and post-ganglionic neurones supplying motor-fibres to the chromophil cells. Elliott was unable to find any nerve-cells in the medulla

of the cat's adrenal, but Müller (1924) found many of them in the human adrenal and he figures a cell with processes ending among the chromophil cells very much as in Selachians. This evidence does not preclude the possibility that in some cases pre-ganglionic fibres run straight through to end on the chromophil cells, but I am of the opinion that this is unlikely.

The interrenal body contains no nerve-fibres except those to the blood-vessels, and presumably its output of secretion is controlled directly by the condition of the blood. In mammals the medulla of the adrenal is richly supplied with nerves which ramify among the cells, whereas the few fibres in the cortex probably pass to the blood-vessels.

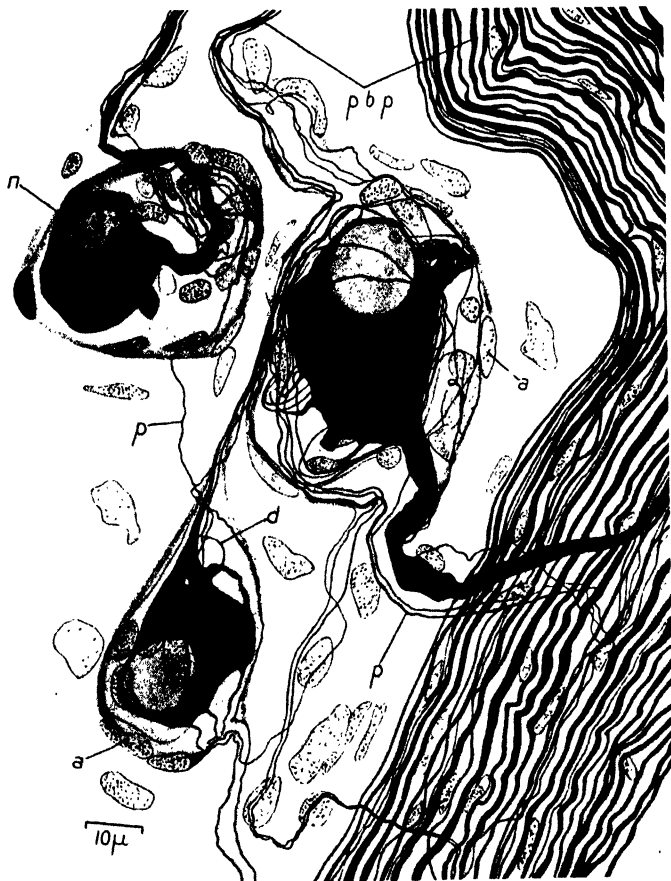
9. Post-Branchial Plexus.

This consists of a network of nerve-fibres lying below the brachial plexus, dorsal to the posterior cardinal veins and lateral to the sympathetic system. It is present in *Scyllium canicula* and *catulus*, but absent from *Rajiformes* and *Heptanchus*. The nerves take origin from the vagus and anterior spinal nerves and consist of medullated fibres similar to those of the rami communicantes. They spread out to form a very complex network which gradually thins out passing backwards and reaches about to the anterior end of the kidney (Text-figs. 5 and 6). The plexus contains many nerve-cells, especially anteriorly, where there may be a post-branchial ganglion (Text-fig. 6), which was noticed by Chevrel.

The cells resemble sympathetic cells in general appearance, many of them being enclosed in nucleated capsules; a typical nest is seen in Text-fig. 20. The cells may be uni-, bi-, or multi-polar, and in the case of the encapsuled cells the synapses appear to be made by means of subcapsular dendrites. In some cases the pre-ganglionic fibres could be seen to make several turns inside the capsule of one cell and then to pass on to another. At the nodes of the post-branchial network there are often single cells which are not enclosed in capsules. Their dendrites make contact with neighbouring fibres without the latter terminating (Text-fig. 21). In such cases it could clearly be seen that there is no actual continuity of substance at the synapse, since the

dendrites were stained yellow-brown and the passing fibres an intense black.

The function of the whole plexus is obscure; possibly it may



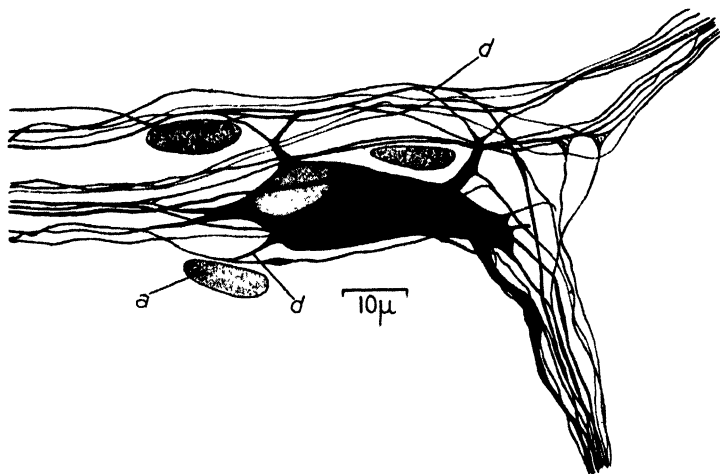
TEXT-FIG. 20.

Scyllium canicula. Nest of cells from post-branchial plexus.
Cajal's method, whole mount. Zeiss. apo. 90, 1·3, camera lucida.

be concerned with the control of the flow of blood in the posterior cardinal sinus, perhaps even to compensate for the absence of accelerator nerves to the heart. Probably the functions are

not so fundamental since the plexus is absent from many Selachians and all Teleostomes, which also lack accelerator nerves.

Besides the motor elements, certain sensory fibres also run in the post-branchial plexus and end in very large sense organs. The fibres run out from the spinal nerves with the rami com-



TEXT-FIG. 21.

Scyllium canicula. Cell of post-branchial plexus. Cajal's method, whole mount. Zeiss apo. 90, 1.3, camera lucida.

municantes (Text-fig. 1), branch several times, and run for considerable distances either with the post-branchial plexus or sympathetic system. They end among the tissues dorsal to the wall of the cardinal vein as elaborate sense organs consisting of whirls of fibres which branch and bear swellings at intervals, the whole organ being surrounded by a nucleated capsule (Text-fig. 22). In the less complex organs of young animals the fibres of the whirls are wholly non-medullated, but in adults (especially of *Scyllium catulus*) many of the outer whirls are medullated (Text-fig. 23). It is not possible to determine for certain whether the branches in the whirls end blindly or whether, and this is probably the case, the whole constitutes

a continuous closed network. Similar organs are present in Rajiformes in which there is no post-branchial plexus. Possibly they function as vaso-proprioceptors, registering the degree of distension of the cardinal veins. Lutz (1930a) found a reflex cardiac and respiratory inhibition after stimulation of



TEXT-FIG. 22.

Scyllium canicula. Sense organ from post-branchial plexus.
Cajal's method, whole mount. Photograph, Zeiss. apo. 90, 13.

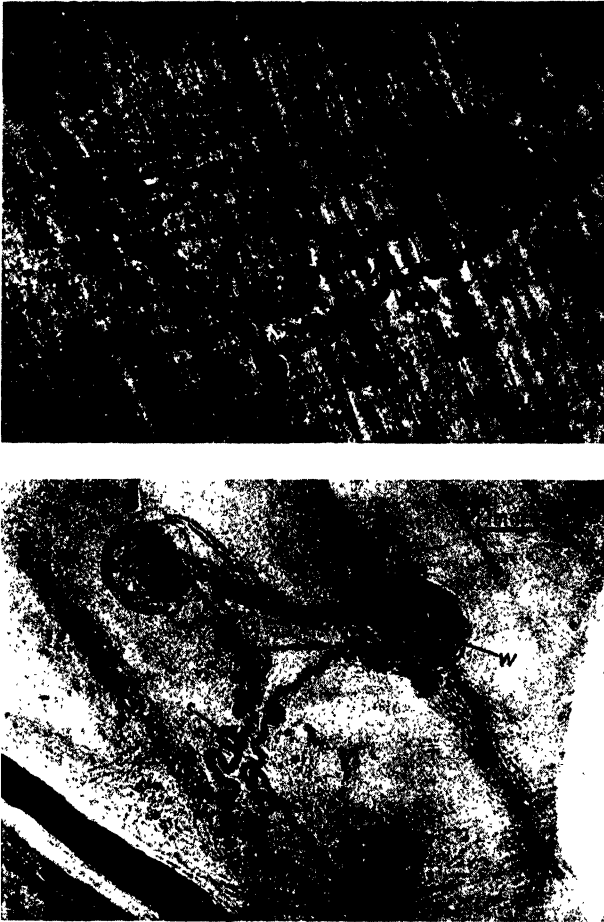
certain fibres which were found to run partly in the vagus and partly in the spinal roots, and it is possible that he was stimulating the sense organs here described. Dogiel (1898) described rather similar sense organs on the arteries of mammals.

IV. INNERVATION OF THE VISCERA.

1. Nerves of the Alimentary Canal.

The smooth musculature of the pharynx is probably innervated from the collections of autonomic cells on the post-trematic rami of the branchial nerves (p. 611), but little is known of their function. The oesophagus and cardiac arm of the stomach are innervated by the vagi and on the right side

the vagus also extends on to the pyloric arm of the stomach. Its hinder branches are very difficult to follow, and in *Scyl-*



TEXT-FIG. 23.

Scyllium catulus. Sense organs from post-branchial plexus.
OsO₄, whole mount. Photograph, Zeiss B.

lium I have never been able to trace any of them posteriorly to the pylorus, but Müller and Liljestrand (1918) report that

in *Raja* there is a branch of the right vagus to the spiral intestine, and Müller (1920) claims to have followed the vagus on to the intestine in developing *Acanthias*.

The most anterior nerves from the sympathetic system to the gut are the right and left anterior splanchnic nerves springing from the axillary bodies. They run with the coeliac artery to be distributed to the oesophagus, stomach, pylorus, and front part of the spiral intestine. The nn. splanchnici medii run with the anterior mesenteric artery to the spiral intestine. They are really compound nerves arising as non-medullated branches from the sympathetic ganglia of several segments (Text-fig. 7). They are much larger on the left side than on the right, where they may be altogether absent. The nn. splanchnici posteriores run as separate strands in the mesentery to the colon and rectum; they arise from the ganglia which are embedded in the kidney.

The innervation of the cloaca is of interest in view of the question of the origin of the sacral parasympathetic system of Tetrapods. The only observations made during the present investigation were on serial sections of late embryos of *Scyllium canicula* and *catulus* (about 25 mm.) and *Torpedo ocellata* (20 mm.) stained with Cajal's method. The walls of the rectum were seen to contain nerve-cells and were innervated from the hinder sympathetic ganglia, but the walls of the cloaca contained no nerve-cells and received twigs directly from two or three spinal nerves. The absence of cells along these nerves shows that they are not part of the autonomic system, but may correspond to the sphincter nerves of *Uranoscopus* (Young, 1931) and to the n. pudendus of mammals (see p. 620).

The functions of the various nerves to the gut have been studied by Stannius (1849), Bottazzi (1902), Müller and Liljestrand (1918), and Lutz (1931). Bottazzi, using *Scyllium* and *Torpedo*, found that stimulation of the vagus caused contraction in the pyloric arm of the stomach and upper part of the intestine. Müller and Liljestrand found that in *Raja* the vagus often caused undoubted increase in the movements of the pylorus and pyloric arm of the stomach, but in other cases the result was an even more definite inhibition of movements in these parts. According to these authors the effect of stimulation

of the anterior mesenteric nerves is to produce anastaltic waves which start at the pylorus and pass forwards along the pyloric arm of the stomach, similar results being obtained by stimulation of the sixth to twelfth ventral, but not dorsal, roots of *Raja*, and of the third to eighth ventral roots of *Squalus*. Bottazzi noticed movements of the rectum after stimulation of the forty-fifth to forty-seventh spinal nerves of *Scyllium*, and Lutz found that stimulation of the posterior splanchnic nerves caused contraction of this organ, but Müller and Liljestrand were unable to obtain any effects by stimulating the middle or posterior splanchnic nerves or the corresponding spinal nerves. It is doubtful how far the results of Bottazzi and Müller and Liljestrand are vitiated by the fact that, although the circulation was kept intact, the viscera were moistened with sea-water which is 'physiologically' hypertonic to the blood and lacks urea.

During the present work some observations were made on the functions of the nerves to the gut, the organs being moistened in all cases with an isotonic solution containing urea (see Young, 1932 a).

The spontaneous movements observed in the intact gut *in situ* and in connexion with the central nervous system were catastaltic waves of contraction in the cardiac stomach and anastaltic waves in the spiral intestine. A pinch in the oesophagus or cardiac or pyloric arms of the stomach caused local constriction but no further effects. A pinch in the intestine caused immediate contraction of the circular muscles behind (orally to) the site of the pinch, and this constriction then often spread orally until the whole of the upper part of the intestine was narrow. The contraction then proceeded to spread backwards from the site of the pinch so that the contents of the intestine were moved towards the anus. In this way the whole intestine became thin and very much elongated and faeces were passed through into the rectum, which contracted in its turn and extruded the bolus. The whole process sometimes occupied as much as 12 minutes, but in other cases was more rapid. Local faradic stimulation of the intestine produced a similar sequence of changes. The functional significance of

these movements in driving forwards the contents of the intestine is obvious.

Stimulation of the vagus caused contraction of the oesophagus and in many cases there was no other observable effect, but often undoubted catastatic movements of the cardiac stomach were induced. This motor effect of the vagus was especially noticeable in cases in which this part of the stomach was already showing spontaneous catastalsis, stimulation of the vagus causing the movements to become much stronger than before. No motor or inhibitory effects posterior to the cardiac end of the stomach were observed after stimulation of the vagus.

Stimulation of the anterior splanchnic nerves, or of the axillary body, or of the anterior rami communicantes, spinal cord, or ventral roots produced a very constant and characteristic effect, namely, a contraction at the pylorus followed by a wave of anastalsis passing up the pyloric arm of the stomach, becoming very deep at the turning and then proceeding forwards up the cardiac stomach, though never reaching more than about half-way along the latter. An exact analysis of the origin of the motor-fibres in the anterior region of the spinal cord was made in a few cases in *Scyllium canicula*. Most of the pre-ganglionic fibres run in the fourth, fifth, and sixth spinal ventral roots, but probably some also pass in the third and seventh nerves.

The movements resulting from stimulation of the middle or posterior splanchnic nerves or of the corresponding sympathetic ganglia or spinal segments were less definite and seemed to depend on the condition of the intestine at the time. However, there is no doubt that the sympathetic supplies motor-nerves to the intestine and stimulation of the middle splanchnic nerves often caused contraction at the point of entry of the nerve, followed by constriction of the whole organ and expulsion of faeces as described above. At other times the effects were less well marked and consisted only in the production of anastaltic waves or in increase in their depth if already present. Similar results followed stimulation of the sympathetic ganglia around the base of the middle splanchnic nerves and of the spinal cord over a considerable length. Since the movements were rather

irregular exact localization was difficult, but the most anterior level after stimulation of which movements of the intestine were observed was that of the fifteenth spinal root, and the most posterior that of the twenty-fifth root. Stimulation of the posterior splanchnic nerves or of the twenty-seventh to thirtieth spinal nerves was followed, in some cases, by movements of the hind part of the intestine and of the rectum.

Müller and Liljestrand did not observe any of these movements of the intestine, and their failure may have been due to the imperfect physiological solutions used or to the state of the animals at the time. For instance, the movements in question could rarely be elicited at Naples with animals kept for a long time in the aquarium, although they were regularly observed on freshly caught animals at Plymouth. The movements of the rectum probably correspond to those observed by Bottazzi, but it is difficult to be certain since in the animals used the anus lies at about the level of the thirty-second spinal nerve and the forty-fifth to forty-seventh nerves stimulated by Bottazzi lie far back along the tail.

It is clear that further and more exact experiments are necessary before the function of the nerves to the gut can be decided with certainty, but it seems that the vagal fibres to the oesophagus and cardiac stomach have motor effects and those to the pyloric stomach sometimes motor, sometimes inhibitory effects. The sympathetic fibres to the pylorus, pyloric stomach, intestine, and rectum are all motor. It is very doubtful whether the vagus has any effect on the intestine.

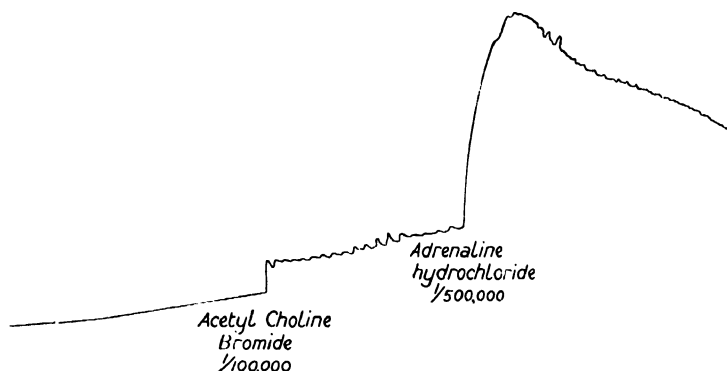
The only observations on the pharmacology of the gut of Selachians appear to be the recent ones of Lutz (1931), who found that adrenaline in rather large doses (1/50,000) caused contraction of the muscles of the pyloric arm of the stomach but inhibition of the rectum (*Squalus* and *Raja*). In a few preliminary experiments on *Scyllium* and *Trygon* I have found that adrenaline in concentrations between 1/50,000 and 1/1,000,000 does, as stated by Lutz, stimulate the pyloric arm of the stomach (Text-fig. 24). Contrary to him, however, I have found that adrenaline also stimulates the muscles of the rectum of *Trygon*. Acetyl choline caused increase in the

tonus of both muscles and the initiation of rhythmic contractions.

2. Innervation of the Urinogenital System.

In each segment the sympathetic ganglia send nerves to supply the oviducts or vasa deferentia, in the walls of which there are complex networks of fibres containing a few ganglion cells. The vagus sends no branches to these organs.

The sympathetic nerves have motor effects on the oviducts.



TEXT-FIG. 24.

Scyllium catulus. Tracing of movements of isolated pyloric arm of stomach, suspended in isotonic solution; pH, 7.6; T, 30° C. Acetyl choline HBr (B.D.H.); adrenaline HCl (freshly made up by neutralization of base). Time in minutes.

Bottazzi (1902) found that stimulation of the spinal cord was followed by contraction of the walls of the oviduct of *Torpedo*, resulting in extrusion of the contained embryos. I have observed contraction of the oviduct of *Scyllium* after faradic stimulation of the spinal cord at various levels and also after stimulation of the nerves which run in the mesentery of the oviduct. The addition of adrenaline hydrochloride was followed by marked contraction of the oviduct suspended in isotonic solution.

No nerves to the kidney tubules were discovered during study

of sections stained with Cajal's method, although many fine bundles of sympathetic fibres run through the kidney tissue to be distributed to the blood-vessels. Control of the secretion of the urine is therefore probably effected only via the blood-vessels, and this is almost certainly the case also in mammals (Cushny, 1926). On the other hand, the urinary sinuses are richly supplied with nerves from the sympathetic system and their walls contain some nerve-cells.

3. Cardiac Nerves.

Stannius (1849) first commented on the absence of sympathetic nerves to the heart of fishes. The question has recently been re-studied by Izquierdo (1930) and Lutz (1930*b*) who were unable to find anatomical or physiological evidence for the presence of accelerator nerves.

I have searched on the anterior and posterior face of the ductus Cuvieri, on the walls of the oesophagus, and in fact in all possible paths without finding any sympathetic branches to the heart. The cardiac nerves, which are known to have an inhibitory action on the heart, arise from the last branchial branch and from the visceral branch of the vagus (Lutz, 1930*b*). They run in the dorsal wall of the ductus Cuvieri and on to the sinus venosus. Here they break up into a great number of branches, forming a complex system round the sinu-auricular opening, where there is a dense network of nerve-fibres and cells. There are no ganglion cells, and only very few nerve-fibres in the walls of the auricle and ventricle.

V. CRANIAL AUTONOMIC SYSTEM.

1. Autonomic Fibres in Branchial Nerves.

The sympathetic system of Selachians differs from that of other vertebrates in that it sends no branches to the head. Chevrel (1887), however, noticed that there is often a connexion between the first sympathetic ganglion and the vagus. The ramus in question was found during the present work in about half of the *Scyllium canicula* examined. It consists of a fine branch leaving the vagus at the level of the third or fourth branchial branch and running with the anterior rami com-

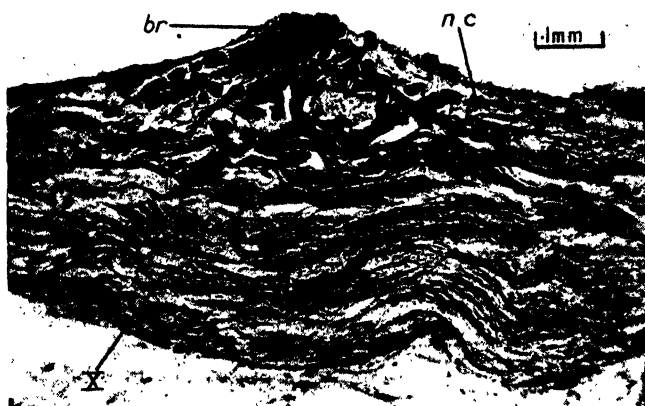
municantes to the axillary body (Text-fig. 6). It is composed of small medullated fibres, apparently similar to those of the spinal rami communicantes, which it very much resembles in position and structure. Since the fibres are medullated it is unlikely that they are post-ganglionic and they are too small to be sensory. Probably they represent a contribution of pre-ganglionic fibres from the vagus to the gastric ganglion. It is certainly curious that the vagus, a dorsal root, should contribute in this way to the sympathetic system which is otherwise connected only with the ventral roots; there is a similar mixture of dorsal and ventral root-fibres in the post-branchial plexus. As the discussion on p. 617 shows, the route of the pre-ganglionic fibres is probably not a question of great morphological importance.

In mammals, besides the post-ganglionic sympathetic supply to the head, there are outflows of visceral motor-fibres in the IIIrd nerve (mid-brain outflow) and in the VIIth, IXth, and Xth nerves (bulbar outflow). The latter outflow consists of fibres of two sorts: those which supply the submaxillary, otic, and parotid ganglia which are developed in connexion with the salivary glands, and the fibres in the vagus which end round the parasympathetic cells in the viscera. As would be expected, the first set of fibres is absent in fish, where there are no salivary glands (Young, 1931). The fibres in the vagus running to the heart and gut are similar in general to the corresponding fibres in mammals: they have been described in more detail above. In addition Norris and Hughes (1920) found collections of motor cells along the post-trematic branches of the facial and branchial nerves of embryos of *Squalus*. I have been able to find these ganglia in adult *Scyllium*, on the hyomandibular branch of the facial and on the post-trematic rami of the glossopharyngeal and vagus, but not on any of the branches of the trigeminal. The cells occur either singly, scattered along the nerves, or in groups from which spring bundles of non-medullated fibres (Text-fig. 25), running presumably to innervate the smooth musculature and blood-vessels of the pharynx.

The size and form of these cells leaves no doubt that they are motor and they very much resemble the sympathetic cells,

being multipolar and having connexions with pre-ganglionic fibres in the form of pericellular baskets or dendritic glomeruli (Text-fig. 26). The sensory cells of the cranial ganglia are of very different form, being larger, bipolar, and only very rarely surrounded by pericellular baskets.

Müller (1920) claimed to have seen motor-cells in the main vagus ganglion of *Squalus* and therefore supposed that there

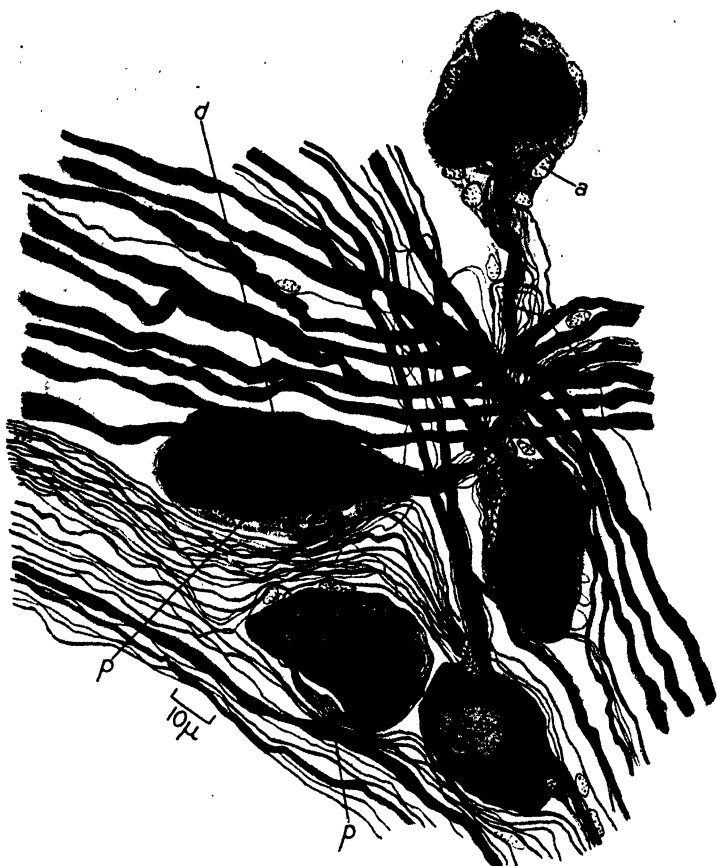


TEXT-FIG. 25.

Scyllium catulus. Motor nerve-cells on post-trematic ramus of vagus. Cajal's method. Photograph, Zeiss A.

were three links in the visceral motor chain. In his sections of embryos he found in the vagus ganglion two types of sensory cells and other smaller cells surrounded by pericellular baskets. Careful examination of serial sections through an 11 cm. embryo of *Scyllium catulus* stained by Cajal's method failed to reveal any motor cells in the ganglion of the vagus or in the other cranial ganglia. Three groups of neurones certainly occur in the vagus ganglion as described by Müller, but the smaller cells are all bipolar and no pericellular baskets could be seen. From his figures it seems possible that the 'baskets' were intracellular neurofibrils and not, as he supposed, the endings of pre-ganglionic fibres.

Several sets of serial sections of the cranial ganglia of adult *Scyllium* were also examined and here again only bipolar sensory cells were found. I conclude, therefore, that the motor



TEXT-FIG. 26.

Scyllium catulus. Motor cells from post-trematic ramus of vagus. Cajal's method. Zeiss apo. 90, 1.3, camera lucida.

cells connected with the dorsal cranial nerves all pass for at least a considerable distance towards the periphery. This removes the evidence for Müller's theory that there are three

neurones involved in autonomic motor chains, and for the present it will be assumed that Langley's view that only two links are involved is correct; however, there is little but negative evidence for either theory, and it would not be surprising to find three-link chains in any part of the autonomic system.

2. Profundus and Ciliary Nerves.

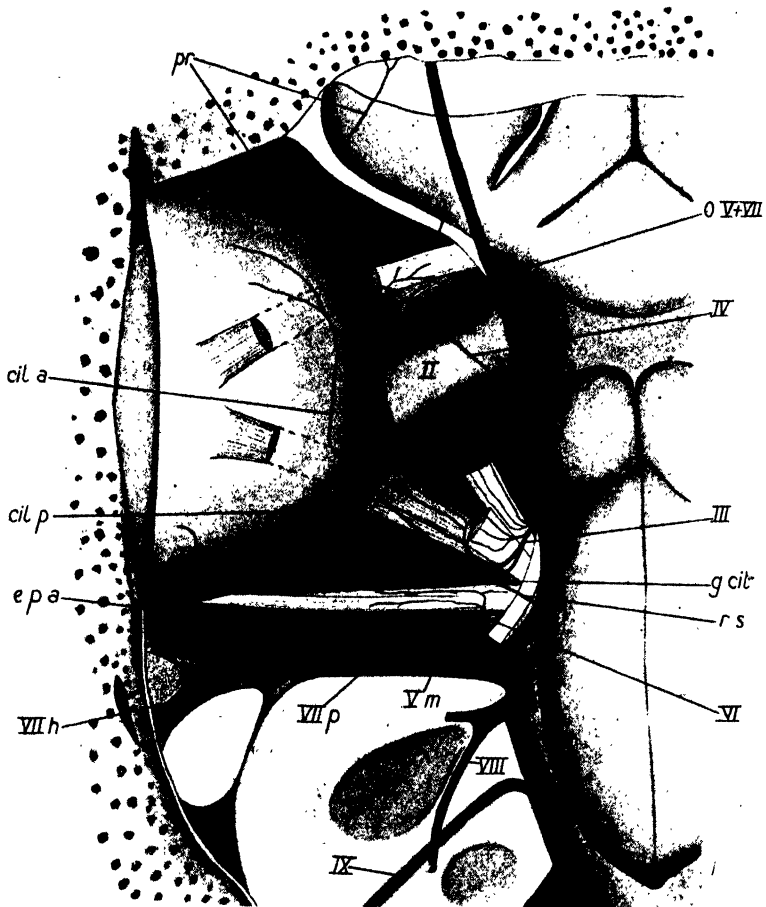
The mid-brain outflow of mammals consists of pre-ganglionic fibres in III which have a synapse in the ciliary ganglion, post-ganglionic fibres passing in the short ciliary nerves to the internal muscles and blood-vessels of the eye. This outflow is well developed in Selachians and its function in regulating the diameter of the pupil has been the subject of a separate investigation (Young, 1933). The whole complex of ciliary nerves differs from that of Teleosts and Tetrapods, first in the absence of a contribution from the spinal visceral-motor (sympathetic) system, and secondly in that the sensory and motor nerves often run separately to the eyeball. The sensory nerves for the eyeball arise in all vertebrates from the nervus ophthalmicus profundus. This nerve is very well developed in Rajiformes and Squaliformes, less so in Scyllioidae, and is usually stated to be absent from Scyllium, where in fact it is present though much reduced.

The simplest condition is seen in *Mustelus*, where there is a typical profundus giving off three branches in the orbit: a n. ciliaris posterior directly after leaving the cranium, a n. ciliaris anterior where it passes between the oblique muscles, these two being sensory nerves, and a third branch, seen by Norris and Hughes (1920), which is motor and consists of non-medullated fibres which bear a ganglion soon after leaving the profundus and then send nerves to the ciliary plexus. The main motor ciliary nerves of *Mustelus* arise from the oculomotor nerve, just after the latter has given off its branch to the inferior rectus. There is a ciliary ganglion at this point which is visible to the naked eye in large specimens and lies so close to the nerve that no separate radix brevis can be distinguished. The short ciliary nerve runs with the ophthalmic artery to the eyeball.

In *Mustelus*, then, the sensory and motor ciliary nerves run separately to the eyeball. In *Scyllium* the conditions are essentially similar but the profundus is very much reduced and the sensory and motor roots join. There are two ciliary nerves (Text-fig. 27) which may be called anterior and posterior though they do not completely correspond with the similarly named nerves of *Squalus* and *Mustelus*. The sensory root of the ciliary complex leaves the skull by a separate foramen just below the foramina for rr. ophthalmici superficiales V and VII, and immediately inside the orbit is joined by a motor root which separates from the oculomotor. The common trunk then divides again to form the two ciliary nerves of which the posterior, where it meets the eyeball, divides up into several branches which pierce the sclerotic. The anterior ciliary nerve runs round in the connective tissue coat of the eyeball, ventral to the anterior rectus muscle; it then divides into two or three branches which pierce the sclerotic and one which leaves the eyeball, re-crosses the orbit between the oblique muscles, and re-enters the cranium by a small foramen in front of the insertions of these muscles. After passing across the dorsal face of the olfactory lobes this nerve is distributed to a small area of skin on the front part of the head. From its position there can be no doubt that this fine branch is the profundus nerve, and it differs from that of *Squalus* or *Mustelus* only in being included for part of its course in the connective tissue around the eyeball. The nerve has often been overlooked in *Scyllium* probably on account of its small size and because, though present in all *Scyllium catulus*, it is sometimes absent from *Scyllium canicula*. In the individuals of this species which have been closely examined the ramus was found to be present on both sides in twelve cases and on one side in one case, whereas in twelve cases there was no profundus on either side. Although later workers have overlooked it, yet the profundus of *Scyllium* was correctly described and figured by Schwalbe (1879), and Gegenbaur (1871) showed that the nerve is also bound up in the eyeball in *Hexanchus*.

The motor nerve-cells connected with the oculomotor nerve

of Selachians are not all collected into a single ciliary ganglion, but are scattered as small ganglia along the course of the nerves



TEXT-FIG. 27.

Scyllium canicula. Drawing of dissection of orbit after treatment with OsO_4 . $\times 3$.

(see Norris and Hughes, 1920). It is important to note that these cells and nerves are not all concerned with the innervation of the smooth muscles of the eyeball, but that many of them

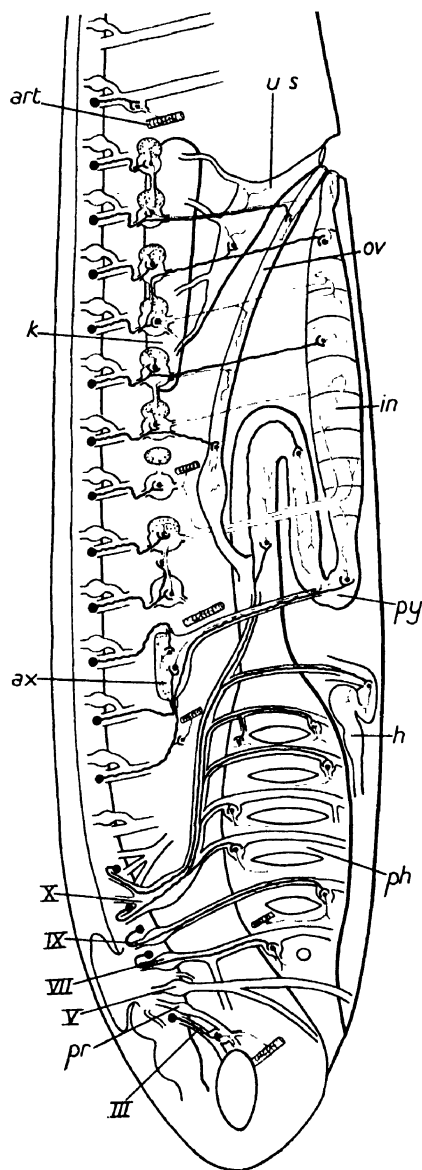
send nerves to the blood-vessels of the orbit. Both in *Scyllium* and *Mustelus* there is a large branch which separates from the motor ciliary root and runs round behind the inferior rectus muscle, where it bears a ganglion from which fine non-medullated fibres run across the orbit to the efferent pseudobranchial and other arteries. Norris and Hughes describe in *Squalus* a branch from this ganglion to the r. palatinus VII; no such branch could be found in *Scyllium*. Other collections of cells were found in *Scyllium* at the point of separation of the motor ciliary nerve from the oculomotor and at the junction of the motor and sensory roots.

In spite of the complexity of details it is clear that there are sensory ciliary nerves to the eyeball from the profundus and motor-nerves from the oculomotor, and that these components may run separately as in *Mustelus* and *Squalus* or together as in *Scyllium*. The visceral motor-fibres in the oculomotor nerve closely resemble the sympathetic fibres of a trunk segment in that (1) they pass through a ventral root, (2) are interrupted in a ganglion whose cells (3) resemble sympathetic cells, (4) they supply motor fibres to the blood-vessels.

VI. PHYLOGENETIC HISTORY OF THE AUTONOMIC NERVOUS SYSTEM.

Langley and Gaskell divided the general visceral motor nerves of mammals into two groups, sympathetic and parasympathetic, characterized by the opposite effects which they have on the heart, gut, urinary bladder, &c. As pointed out by Goodrich (1927, 1930), these systems are morphologically very difficult to characterize, since while the sympathetic fibres run only via the ventral roots, the parasympathetic fibres pass partly through the VIIth, IXth, and Xth cranial nerves which are dorsal roots, and partly through the oculomotor and pelvic nerves which are ventral roots.

Now it is conceivable that there were originally two complete sets of antagonistic fibres passing out in the dorsal and ventral roots respectively, but Young (1931) found no evidence of this in Teleosts, and in *Amphioxus* all the fibres run to the



TEXT-FIG. 28.

Diagram of autonomic nervous system of *Scyllium*. Pre-ganglionic neurones black, post-ganglionic neurones red.

viscera through dorsal roots and this was presumably the original condition. The problem is therefore 'how have "sympathetic" neurones come to be connected with the central nervous system through ventral roots?' (Goodrich, 1927). Hoffmann (1900) pointed out that 'sympathetic' (sens. strict.) ganglia are only formed in those segments in which the dorsal and ventral roots join, and Goodrich suggested that the pre-ganglionic fibres come to run through the ventral roots in the segments in which the roots join, but remain in the dorsal roots in those segments (VII, IX, and X) in which the roots remain separate.

This condition is very well illustrated in the Selachians in which there are visceral motor fibres in almost every segment from the front of the head to the anus, running through the dorsal roots (VII, IX, and X) when they remain separate and through the ventral roots where they join (III, spinal segments). It is interesting that in *Squalus* and *Mustelus*, in which the sensory and motor ciliary roots only partly join, Norris and Hughes (1920) found motor fibres running out in the profundus.

There is very little evidence in fish of a differentiation into functionally antagonistic sympathetic and parasympathetic systems. In Selachians the vagus supplies motor fibres to the pharynx, oesophagus, and cardiac stomach, while the sympathetic ganglia send motor fibres to the pyloric stomach and to the whole of the intestine. The only place in which the vagus and sympathetic nerves overlap is the pyloric stomach, and here there is some evidence that the vagus inhibits the movements whereas the sympathetic stimulates them (p. 605). In Teleosts Müller and Liljestrand (1918) report that stimulation both of the vagus and of the splanchnic nerves caused movements of the stomach, and I have been able to confirm this in *Uranoscopus*, in which, as in Selachians, the whole of the intestine receives motor fibres from the sympathetic ganglia (unpublished).

It is significant that both in Selachians and in mammals the structure of the neurones in all the autonomic ganglia is similar, whether they are connected with dorsal or with ventral roots; and the fact that both the vagus and the spinal nerves

contribute pre-ganglionic fibres to the gastric ganglion and post-branchial plexus also indicates that all the autonomic fibres belong to a single system. It would be very interesting to discover a case in which fibres having similar functions passed partly through dorsal and partly through ventral roots. It is possible that such a condition is found in the case of the vasomotor fibres of Selachians, since some of them probably run through the vagus (Hoffmann, 1900). If this is so we have a single set of vasomotor fibres all down the body, some passing through dorsal and some through ventral roots.

Probably, therefore, in the earliest Chordates there were visceral fibres in every segment, passing, as in *Amphioxus*, through the dorsal roots. When the dorsal and ventral roots became joined the motor fibres came to run out in the ventral roots, forming the sympathetic system, but remained in the dorsal roots in the cranial region, where the roots remained separate.

The arrangement was at first segmental, nerves running to the viscera in every segment as in the posterior abdominal region of Selachians. At this stage probably all the nerves had motor effects on the organs which they innervated. Later, the vagus spread farther and farther back along the gut, as it still does in the course of ontogeny of modern forms, overlapping the sympathetic innervation. At first the vagus and sympathetic both had motor effects on the organs which they innervated together, as they still do in the stomach of Teleostomes and Amphibia; but later, possibly as a result of changes in the terminal apparatus (see Brown and McSwiney, 1932), the two came to have reciprocal effects of excitation and inhibition, giving the very efficient mechanism which is found in Amniotes for the control of the viscera. At the same time the sympathetic system became specialized, the segmental ganglia becoming connected by chains to allow of more efficient correlation, while the numerous segmental nerves to the viscera were replaced by a few splanchnic nerves.

The sacral parasympathetic system is not accounted for in this hypothesis. It is absent in Selachians and Teleosts (Young, 1931), but appears in the earliest Tetrapods (Langley and

Orbeli, 1911) probably in connexion with the formation of a cloacal bladder. Possibly the sacral outflow was derived from the n. pudendus, which is present in both Selachians and Teleosts, by the migration along it of motor cells when it took on the function of innervating the smooth musculature of the cloacal bladder.

Langley and Gaskell placed considerable emphasis on the gaps between the visceral motor fibres of the cranial and thoracico-lumbar sets and between the latter and those of the sacral nerves. Langley and Orbeli found similar gaps in Amphibia and they also appear in Selachians and Teleosts between the cranial and spinal sets of visceral motor fibres. However, there does not seem to be any special morphological significance attached to these gaps, rather we must consider the autonomic nervous system as consisting fundamentally of a single segmental series of motor fibres to the viscera.

Our knowledge of the autonomic nervous systems of the more primitive vertebrates remains fragmentary, but such as it is it seems to show that the sympathetic and parasympathetic systems of mammals, with their complex balance of excitation and inhibition, are recent developments. They do not represent two morphologically distinct systems but are specializations within an originally simple visceral motor system whose neurones left the central nervous system via the dorsal roots. Further research is now being made into the course and functions of these fibres in Cyclostomes, Fish, and Amphibia in order to determine whether this view is correct.

VII. SUMMARY.

1. The rami communicantes of Selachians contain only pre-ganglionic fibres; there are no recurrent grey rami and therefore no sympathetic nerves to the skin, chromatophores, or somatic muscles. This probably accounts for the absence of the sympathetic from the head and tail regions.

2. In accordance with (1) it was found that cutting of the spinal nerves produced no local colour changes in the skin, neither was adrenaline found to have any action on the chromatophores.

3. There are no long pre- or post-ganglionic pathways in the sympathetic and therefore no true sympathetic chains, though the ganglia of adjoining segments are sometimes connected. The arrangement is thus more nearly segmental than that of Teleosts or Tetrapods.

4. No sympathetic ganglia were found in the tail of adult *Scyllium* or *Torpedo*, but in embryos of these forms scattered motor neurones were found in connexion with the caudal blood-vessels.

5. Stimulation of the vagus caused movements of the cardiac stomach, of the anterior splanchnic nerves movements of the pylorus and pyloric stomach. Stimulation of the middle and posterior splanchnic nerves caused movements of the intestine, colon, and rectum. Pinching the intestine evoked a characteristic progressive reflex contraction, ending in the extrusion of faeces.

6. The posterior suprarenal bodies differ in the two sexes, those of the male being much the larger, although the number of cells giving the chrome reaction is the same in both.

7. The suprarenal tissue is very plentifully supplied with post-ganglionic fibres, which could be seen actually in connexion with their cell-bodies. The hypothesis of Elliott that the chromophil cells themselves represent post-ganglionics is therefore disproved in this case.

8. The structure of the autonomic neurones is described in detail, especially the methods by which contacts are made between them.

9. No motor cells were found in the vagus ganglion of embryo or adult *Scyllium*, but they do occur on the post-trematic rami of all the branchial nerves.

10. A small profundus nerve was found to be present in *Scyllium*, though not in all individuals.

11. There is little evidence for the existence in fish of functionally antagonistic sympathetic and parasympathetic systems, and it is suggested that these systems in Tetrapods represent specializations within a single segmental set of visceral motor fibres, running primarily through the dorsal roots but coming to pass through the ventral roots in those segments in which the roots join.

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Habits, Structure, and Development of *Spadella cephaloptera*.

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With Plates 34-8 and 5 Text-figures.

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1. INTRODUCTION.

DURING the summer of 1930 Prof. E. W. MacBride, F.R.S., sent me to Plymouth to work out the Anatomy and Development of *Sagitta* with a view to giving a complete account of the species common on the English coast and to verify some of the interesting points which had arisen with regard to its anatomy. In this attempt I was confronted with more than one practical difficulty: specimens of *Sagitta* cannot be kept alive under artificial conditions; in fact most of them die even before the trawler reaches shore. Their eggs are found in the tow-net

only during certain seasons of the year, and even then not in sufficient numbers. As the development of *Sagitta* is very rapid the earlier stages are very difficult to obtain, and these facts probably explain why there are only a very few papers dealing with the development of *Sagitta*. During the summer *Sagitta elegans* and *Sagitta setosa*, which are commonly found in the neighbourhood of Plymouth, are usually immature and every attempt to keep them alive failed, but while engaged in my work on *Sagitta*, I was informed by Mr. Smith, the laboratory assistant of the Marine Biological Laboratory, that *Spadella cephaloptera* is found in large numbers in a few of the tanks of the laboratory and in the tidal pools of the Plymouth Sound. As it will be shown in the following pages, *Spadella*, being a coastal form, is very hardy and thrives admirably under laboratory conditions. It reproduces all the year round and the eggs can be easily collected and reared. It is therefore best suited as a convenient and easily obtainable type for purposes of study and future research.

There are only very few scattered and incomplete accounts of *Spadella* in the literature on the group Chaetognatha. The genus *Spadella* was created by Pagenstecher in 1854. O. Hertwig (1880) and Grassi (1883) have both given short accounts of the species *Spadella cephaloptera*; Busch, and later Gourret (1884), gave an elaborate account of the habits and structure of *Spadella marioni*, but his work is very incomplete and to some extent inaccurate. The characters of the two genera *Sagitta* and *Spadella* are very distinctive when the more specialized species of the two genera are compared, but there seem to be some intermediate forms, which do not possess all the characters of either of the genera, but combine some of each. *Spadella marioni*, described by Gourret, is one such species so intermediate in structure between the two genera that Gourret was led to doubt many of the observations of earlier workers. It is also interesting to note that the development of *Spadella* has never before been worked out. This paper therefore aims to give a complete account of the habits, structure, and development of *Spadella cephaloptera*, which by its habits is better suited and more

easily accessible to the student of zoology than the allied genus *Sagitta*.

This work was carried out in the Huxley Research Laboratory of the Imperial College of Science and Technology and in the Laboratory of the Marine Biological Association at Plymouth. For material and facilities for conducting rearing work I am indebted to Dr. E. J. Allen, F.R.S. For suggesting this line of investigation and for the greatest help and encouragement I wish to express my deepest gratitude to Prof. E. W. MacBride, under whose direction this work has been carried out. I am indebted to Mr. H. R. Hewer for the helpful suggestions and constructive criticism which he has always willingly given me throughout the period of my work at the Imperial College; and also to Prof. E. S. Goodrich for valuable suggestions, corrections, and the loan of sections.

2. HISTORICAL RÉSUMÉ.

The earliest notice of the animal belonging to the phylum Chaetognatha was given by Slabber (1781), who named it *Sagitta*. This notice, however, seems to have been forgotten until Quoy and Gaimard rediscovered the animal in the Straits of Gibraltar in 1827 during their second voyage round the world. The species discovered by them was named *Sagitta bipunctata* and placed among the Zoophytes. In 1845 D'Orbigny discovered three new species and classified them with Pteropod molluscs. Other species have since been noticed and more or less accurately described by Scoresby, Fobers, Darwin, Wilkins, Huxley, and Busch. The curious and interesting structure of the genus, its extreme transparency and great abundance rendered it an attractive subject for intensive research. In 1844 Krohn made a detailed study of the general structure and anatomy of *Sagitta bipunctata* and for the first time described the nervous system, in which he discovered great similarity with the nervous system of Mollusca. The general body-cavity was found by Leuckart and Pagenstecher in 1854; the latter also created the order Chaetognatha containing two genera, *Sagitta* and *Spadella*, and placed it between Annelids and Nematodes.

In 1856 Meissner suggested that the Chaetognatha must be placed near the vertebrates, an opinion which was later supported by Haeckel. The anatomical researches of Claparède (1863) and Leydig (1864) brought to light many details which finally led to the work of Kowalevsky (1871) who made the first important study of the development of the animal and also described the nervous system. Later O. Hertwig (1880) made a careful study of the histology and early stages of development and also a systematic survey of the different species of the two genera till then known. In 1883 Grassi carried out an intensive research on the Chaetognatha of the Gulf of Naples with special reference to the cephalic muscles, the structure of the intestine and fins, and spermatogenesis.

In more recent years the results of the various marine scientific expeditions have added greatly to the number of species, whilst the group itself has suggested many important problems of wider scientific interest. Kofoed (1907) collected statistics from all the published accounts, especially that of Fowler (1905), regarding the geographical distribution of the different species of *Sagitta*, and showed their special interest bearing on the relation of isolation to the origin and preservation of the species. Ritter Zahony produced a number of important papers on the anatomy and systematics of the group and pointed out that the name 'Krohnia' had previously been used for another group of animals and therefore suggested the new name 'Eukrohnia' for the third genus. Michael (1911) studied the vertical distribution of *Sagitta* in the Californian region and gave a detailed account of the classification of the different species, and Stevens (1910) published several papers on the spermatogenesis, oogenesis, and reproduction of *Sagitta bipunctata*.

During this time the only important works on the embryology of the group was by O. Hertwig (1880) and Doncaster (1902).¹ The work of Doncaster on the development of *Sagitta* with special reference to the formation of the transverse septa, head cavities, oviduct, sperm duct, &c., is the most significant con-

¹ See also Bütschli (Zeit. wiss. Zool. v. 23, 1873), and Elpatievsky (Anat. Anz. v. 35, 1905). [Editor.]

tribution to our present knowledge of the group and to the solution of the problem of its affinities.

During recent years no work of importance has been done on *Spadella*, and its embryology and development has never been worked out; this is probably because *Spadella* is not so abundant either in numbers or in species as *Sagitta* and has never before been recorded on the English coast.

3. HABITS AND HABITAT.

The eastern shore of the Plymouth Sound is formed of a platform of rocks ending seawards in an abrupt edge. During low tide the rocks are partly exposed, but during high tide they are covered with about two or three feet of water. The surface of the rocks is clothed with a dense growth of sea-weeds and studded with irregular pools and channels. These protected pools and shallow bodies of water, which are not greatly disturbed by the waves, are the natural habitat of *Spadella cephaloptera*.

While the majority of the species of *Spadella* and almost all the species of *Sagitta* are free-swimming and are captured in large numbers during certain seasons of the year, the habits of *Spadella cephaloptera* are peculiar and interesting. In the natural condition it is always found adhering to the smooth surface of sea-weeds and probably small pebbles and rocks by the adhesive papillae on the ventral surface of the caudal region. It is interesting to note that *Spadella cephaloptera* was never captured in the open sea or from the deeper parts of the harbour. In fact during the three months of my work at Plymouth on one occasion only was a single specimen obtained while tow-netting in the harbour. The greater part of my collection was made from one of the tanks of the laboratory, in which they are found in considerable numbers. During the day they swim to the glass side of the tank and remain attached to the surface, when they can easily be detected from outside and captured with the aid of a long pipette. Sometimes even the strength of the current of water entering the pipette was insufficient to dislodge them from the surface of the glass. Specimens were also obtained from the tidal pools

mentioned above, by taking bits of sea-weed and shaking them vigorously in bottles containing sea-water. It is also possible to capture specimens by scraping up the soft layer of bottom deposit of the tidal pools and stirring it in finger-bowls. When the seaweeds are disturbed these animals detach themselves and dart into the bottom mud, where they remain partly buried till the surroundings become calm again.

In all places where *Spadella cephaloptera* has been previously obtained, the conditions of the locality seem to be the same as those which have been observed at Plymouth, except in the case recorded by Claparède (1863). It was first observed in the Messina harbour by Hatschek and O. Hertwig among sea-weeds in shallow places. Busch (1851) collected *Spadella* in the Orkney Islands from the bottom mud at a depth of 8-12 fathoms, while Claparède obtained large quantities of material by tow-netting in the bay of Normandy, and by placing tow-nets against receding currents of tidal pools. On the basis of this observation he maintains that *Spadella cephaloptera* is pelagic like other Chaetognatha. However, it seems probable that the occurrence of this species in the deeper parts of the bay could only be regarded as a rare phenomenon, brought about by some extraordinary condition, their natural habitat being the shallow regions near the coast. From the account of Claparède one might infer that there was a sporadic increase in numbers in that particular season, and that the strong currents of the receding tide swept them down into deeper water; how else could one account for the fact that he was able to collect them in large numbers by placing tow-nets against the currents of tidal pools? The observations of Claparède cannot therefore be regarded as representing the natural habits of *Spadella cephaloptera*. Careful observations in the tanks of the Marine Biological Laboratory has convinced me that *Spadella cephaloptera* is not adapted for pelagic life. These animals always remain attached to the sides of the tank or partly buried in the bottom mud. When disturbed they detach themselves and dart through water only to find some other place to rest. They do not swim except for a fraction of a second, and if during that time they do not reach some suitable

object, sink to the bottom without any additional effort. Their inability to remain suspended in water, or swim for a considerable time, together with their special adaptation for adhesion, sufficiently prove that they have a more or less sedentary habit. In the resting state the trunk is lifted upwards at an obtuse angle with the tail, so that the head is raised considerably above the surface of the object of attachment. Their locomotion is by a series of jerks. By this process they are able to move rapidly on the surface of the object on which they rest or through water. This mode of progression is mainly effected by muscular contractions of the trunk and tail, during which the fins act as balances, the fins by themselves being incapable of independent movement.

Spadella cephaloptera feeds mainly on small Crustaceans. When reared in the laboratory they were fed with live Harpacticids, which are found abundantly in the tanks. The prey, when it approaches close to the head, is seized between the prehensile spines and swallowed whole. Cannibalism has been observed on many occasions, but invariably the victim is a young one, which has not grown to more than half the size of the adult. Whenever a prey is caught the head is swallowed first, and if the victim is too long to be swallowed whole, the posterior part is cast off. This has been noticed only during cannibalism when the victim happened to be too long.

When captured specimens were kept in finger-bowls containing sea-water they remained attached to the surface of the glass, very often on the side which received more light, and if the water in the vessel was left unchanged for more than a day, they became restless and frequently floated on the surface of the water with the ventral side upwards. They are very hardy and remained active even when the salinity of the water was reduced to 80 per cent. of the normal. When kept in sea-water of 60-70 per cent. of normal salinity they rested motionless and became indifferent to any kind of disturbance, but revived immediately when transferred to normal sea-water. This ability to withstand reduced salinity may be cited as a factor of some importance to their established mode of life. They are coastal inhabitants of shallow bays and sounds, the waters of which are mixed with

a greater or less amount of fresh water from the rivers which may open into them.

4. EXTERNAL CHARACTERS.

Spadella cephaloptera is the smallest Chaetognath known, a fully grown adult measuring only about 4.25–5 mm. in length. The body is opaque and certain areas are coloured with traces of brown pigment. Owing to the thinness of the ectoderm most of the internal organs are visible under the microscope in the living condition, but the main ganglia are seen only in stained preparations. To the naked eye and under low magnifications the body has the form of a short ribbon divisible into four regions: a thick rounded anterior head, a flattened collar region or neck, which is really part of the trunk, the trunk proper, and the caudal region.

The head has the form of a short truncated cone with a broad base and rounded apex. It carries on each side near the base a bundle of prehensile spines and near the apex a row of short conical setae. The mouth is situated in an antero-ventral position at the bottom of a shallow ventral depression, the vestibule. The main mass of the head is formed of a number of strong muscles, which give the characteristic contour, and regulate the various movements in connexion with food capture. The head is compressed dorso-ventrally, the dorsal surface being flat and the ventral surface convex, and is covered over by a reduplication of the ectoderm called the hood, which in the resting condition forms a sheath enclosing the prehensile spines and setae. It is attached round the neck and to the dorsal surface of the head, the dorsal part extending to the anterior end of the head, where it dips downwards and forms the anterior boundary of the vestibule. The hood ends ventrally behind the mouth, round which it leaves a wide oval opening, so that when the hood is drawn over the head, only the vestibular pit and the mouth are left uncovered. When the animal is about to catch prey, the hood is drawn backwards exposing the anterior half of the head. In this condition (figs. 17 and 18, Pl. 36) the hood is seen as a thin transversely circular sheath covering only the posterior part of the head and the base of the prehensile spines. On the dorsal surface of the head behind the cerebral ganglion

are the two eyes, easily recognizable as two blackish spots. There is also a pair of club-shaped stumpy tentacles, one on each side, placed laterally near the base of the prehensile spines. They are formed of vacuolated cells and do not seem to be capable of contraction. When a living specimen is examined under the microscope the tentacles are closely pressed backwards to the sides of the head, and even in the preserved condition, only the greatest care in mounting shows the tentacles distinctly. This may perhaps account for the remark of Giard (1875) that tentacles are not present in *Spadella cephaloptera*.

In the fossil *Chaetognath*, *Amiskwia sagittiformis* (Walcott, 1914), the tentacles are very strong and probably served as important tactile organs, but the nature and size of the tentacles in *Spadella cephaloptera* leads one to conclude that they are only the vestiges of once functional organs, which do not serve any useful purpose and which are completely lost in the more advanced pelagic forms.

The head passes insensibly into the slightly broader neck. This region, though not sharply differentiated from the trunk, yet shows certain distinctive characteristics of its own. It is much compressed and formed of convex dorsal and ventral halves, meeting laterally at an angle (fig. 7, Pl. 34). The sides are formed of loose parenchymatous tissue, but on the ventral surface the ectoderm is thin and smooth. The corona ciliata (fig. 7, Pl. 34, and fig. 18, *c.c.*, Pl. 36) is placed on the dorsal side of the neck. It has the form of a conspicuous oval ring of deeply-staining ciliated cells extending from side to side. Its longest diameter is at right angles to the long axis of the body, a factor of some importance in distinguishing this species from *Spadella draco*, in which the longest diameter of the corona ciliata is parallel to the long axis of the body.

Behind the neck the trunk is fairly even in outline and circular in cross section. It widens slightly at its posterior end, where the female genital openings are situated. The alimentary canal extends the whole length of the trunk and the anus opens ventrally in the median line, at the extreme hind end of the canal in front of the septum separating the trunk from the

caudal region. The visceral ganglion is placed in the ventral wall of the trunk about a third of its length from the head. The two ovaries are placed one on each side of the intestine, extending forwards nearly to the posterior limit of the visceral ganglion. In a mature specimen each ovary is filled with six to eight large opaque eggs, which are clearly seen even in the living condition. The female genital openings are placed laterally one on each side of the anus and are surrounded by conspicuous cement glands.

The tail, though separated by a transverse septum from the trunk, does not constitute a distinct segment (Meek, 1928). There is a median longitudinal septum running the entire length of the tail and dividing it into right and left chambers, which in a mature specimen are completely filled with masses of sperm mother cells. The tail narrows gradually from before backwards and ends in a pointed apex, which bears the horizontal caudal fin. This caudal fin has the form of a triangle into whose truncated apex the tail is inserted as a re-entrant angle. Immediately in front of the anterior end of the caudal fin there is a pair of oval swellings, the seminal vesicles (fig. 16, *ves.sem.*, Pl. 35) which carry the male genital openings. The single lateral fin on each side extends the entire length of the tail from the posterior end of the trunk to the seminal vesicles, which therefore seem to be wedged between the lateral and caudal fins.

In stained preparations of whole mounts the ectoderm is seen to carry a large number of clusters of deeply-staining cells, which show a certain regularity of arrangement on the body and fins.

5. INTEGUMENT.

The epidermis varies in thickness in the different regions of the body and is formed of a single or double layer of cells, which are hexagonal or pentagonal. A small oval nucleus is placed at the deeper end of each cell and the protoplasm carries a diffused brownish pigment, which gives the opaque colour to the body-wall. Viewed from the surface, the edges of the cells have an irregular outline which fits closely into adjacent cells. The epidermis is covered on the outside by a layer of cuticle. On the sides and ventral surface of the head the epidermis is comparatively thick, but on the trunk and tail it is very thin.

The cells of the epidermis are modified in certain regions into clusters of ciliated or glandular cells. The ciliated cells occur in the wart-like prominences on the body and in the corona ciliata. Each wart (Text-fig. 1 B) has the form of a small cone projecting slightly above the level of the surrounding epidermis, and with a pit at its apex. A tuft of long cilia projects out through the external opening. These tufts of cilia are in constant vibration when the animal is resting.

The wart-like prominences described above are called the ciliated pits. They show a regular arrangement on the body and their number seems to be almost invariable in the specimens examined. There are ten ciliated pits on the head, arranged in pairs, the members of each pair occurring one on each side of the median line. In front of the mouth there are two pairs, one dorsal and the other lateral. The lateral pair is the most anterior one and the dorsal pair occurs slightly behind it, while in some specimens they both lie in the same vertical plane. In the region of the mouth there is a ventro-lateral pair and in the hind end of the head there are two pairs, one lateral and the other ventro-lateral, situated almost in the same transverse plane.

On the trunk there are six pairs of ciliated pits placed laterally, five unpaired dorso-median pits, and many smaller pits. The lateral pits are more prominent and are placed almost equidistant from each other. Between each successive pair of lateral pits there occur three pairs of smaller pits; of these, one is dorso-lateral, one ventro-lateral, and one dorsal. On the tail the ciliated pits are restricted to the dorsal surface of the body, and to the base of the lateral fins. There is an unpaired dorso-median row of five pits, five pairs of dorso-lateral, and five pairs of ventro-lateral pits. The dorso-lateral and ventro-lateral pits occur on each side above and below the bases of the lateral fins. On the dorsal surface of the lateral fins there are two pairs of pits, the first placed near the outer border of the broader part of the fin and the other near its posterior end. On the tail-fin the pits occur only on the dorsal surface. There are three on each side of the insertion of the tail into the fin, and five near the hinder border of the fin, arranged in a semicircular row.

The cells composing these invaginations are larger and stain

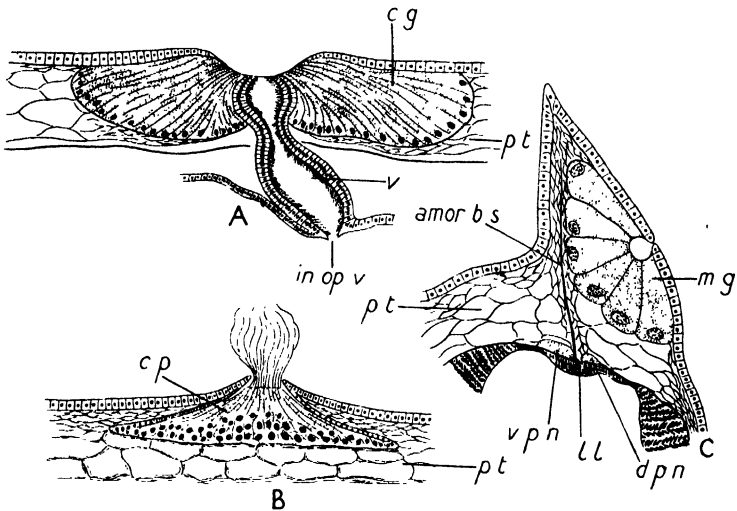
deeper with haematoxylin than the normal ectoderm cells. Each pit has the form of a narrow-necked conical flask which has been much compressed, both dorso-ventrally and laterally. The long axis of the oval base of the pit is parallel to the long axis of the body. The external opening is surrounded by ordinary epidermal cells, while the cavity itself is lined with modified cells. Of these, the cells forming the roof are small and non-ciliated and those in the base are large and ciliated.

The dorsal corona ciliata (fig. 7, Pl. 34, and fig. 18, *c.c.*, Pl. 36) is formed of cells which are identical with those found in the ciliated pits. The body of the ring is five or six cells in thickness and is implanted in the epidermis, the cilia alone projecting freely on the surface. It has been suggested by previous workers that the corona ciliata is an olfactory organ, an opinion which was based only on conjecture. When selected specimens are placed in water of 70 per cent. of normal salinity, they become inactive and remain attached to the sides of the finger-bowl. In this condition it is possible to test the sensitive reaction of the different parts of the body by directing a small jet of water on the surface of the animal, and it has been found that the animal endeavours to shift its position only when the jet is directed on the corona ciliata. It has also been observed that during sperm-transference two animals approach close together with the head of the one pointed towards the tail of the other. In this position they lift their heads simultaneously at intervals, during which the collar regions of the two animals alone touch. It is, therefore, almost certain that the corona ciliata is the most sensitive region of the body and that it functions as a tactile organ, which detects any disturbance in the surrounding water. Since the ciliated pits also have an almost similar structure, it is probable that they are localized secondary sense organs, which serve the same purpose as the corona ciliata.

The chief glandular modifications of the epidermis are the cement glands and the mucous glands. The cement gland (Text-fig. 1 A) is placed round the external opening of the female genital organ. It is a large oval gland formed of long narrow cylindrical cells with basal nucleus and fine glandular contents,

and covered by a thin layer of epidermis on the other side. The cells converge round the opening of the vagina and they secrete the cement substance only when the eggs are passing out. The cement substance forms a thin covering round the egg with a short stalk at one end. The stalks of all the eggs, laid at the same time, are held together in a cluster, which is attached to some foreign object.

The mucous glands (Text-fig. 1 c) occur in the dorso-lateral



TEXT-FIG. 1.

Modification of the epidermis. A. Cement gland. B. Ciliated pit. C. Mucous gland. *amor.b.s.*, amorphous basal substance; *c.g.*, cement gland-cells; *c.p.*, ciliated pit cells; *d.p.n.*, dorsal parietal nerve; *in.op.v.*, inner opening of the vagina; *l.l.*, lateral line; *m.g.*, mucous gland-cells; *p.t.*, parenchymatous tissue; *v.*, vagina; *v.p.m.*, ventral parietal nerve.

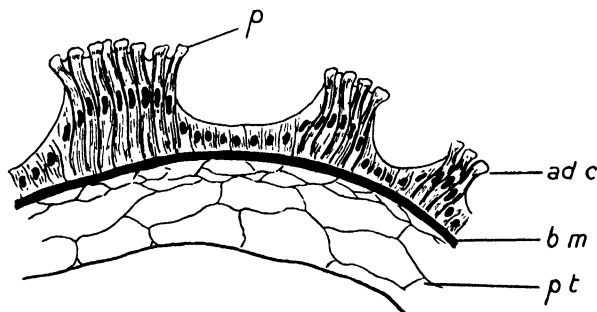
angle between the lateral fins and the sides of the tail. There are four successive rows of glands on each side of the tail, each row placed between successive lateral ciliated pits and formed of four or five adjacent glands arranged in a linear order, parallel to the long axis of the body. Each gland is hemispherical in shape, with the convexity facing inwards. It is covered with a thin layer of epidermis with a small aperture in the middle. This aperture opens into a shallow cup-shaped

depression, which is bounded by the apices of the large conical glandular cells. These cells are similar to the cells of the cement gland, except that they are larger and fewer than the latter. The mucus secreted by them is poured into the cup-shaped depression, from which it exudes through the aperture in the epidermis. When examined in sections these cells appear to have finely granular contents and generally remain unstained. These mucous glands are probably identical with the subdermic glands, described by Grassi (1883) in *Sagitta claparidae*, but hitherto their general disposition and function had not been properly described and elucidated.

On the ventral surface of the tail the epidermis bears clusters of adhesive papillae. These were first discovered by Busch (1851) and later described by Hertwig (1880) and Grassi (1883). The papillae were regarded as clusters of differentiated cells situated outside the ordinary ectoderm, clearly distinguished from the subjacent cells of the normal epidermis. This opinion is not in agreement with the present observations. Text-fig. 2 shows the structure of the papillae and their relation to the surrounding epidermis. The cells of the epidermis at intervals become larger and elongated, and form wart-like swellings on the surface. Each wart is formed of six to ten modified epidermal cells (*ad.c.*), and each cell is drawn out into a finger-like process with a swollen tip, called the papilla (*p.*). The nucleus is situated in the middle of each cell, and it will be noticed that a subjacent layer of epidermis does not exist, each cluster of papillae being continuous at the sides with the surrounding layer. The papillae are more numerous in the anterior half of the tail than the posterior, and they do not occur behind the region of the seminal vesicles. In the anterior end of the tail six to eight clusters can be counted in a transverse row, while posteriorly they diminish in number and become smaller in size. These papillae are the chief organs of adhesion. Their swollen tips are sucker-like and function like the tube feet of Echinoderms, in securing a firm attachment of the animal to foreign objects.

The basement membrane is thin and structureless, and forms a continuous layer below the epidermis. It is slightly thicker in the collar region and below the glandular areas around the

mouth. Between the basement membrane and the bundles of muscles in the trunk and tail there is a layer of "parenchymatous" tissue. In the sides of the body it is highly vacuolated, but in the dorsal and ventral regions it exhibits a close reticular structure. Transverse sections through the different regions of the trunk and tail show this layer as a network of fine strands. In the collar region the parenchymatous tissue is confined to the right and left sides (fig. 7, *p.t.*, Pl. 34), being very inconspicuous in the dorsal and ventral regions, while in the trunk



TEXT-FIG. 2.

Adhesive cells on the ventral epidermis of the tail. *ad.c.*, clusters of adhesive cells; *b.m.*, basement membrane; *p.*, papilla; *p.t.*, parenchymatous tissue.

and tail it forms a continuous layer of varying thickness outside the muscular layer (figs. 9-16, *p.t.*, Pl. 35).

The Fins.—The external appearance and position of the fins have already been described. In the living animal the fin is quite transparent and the rays are seen in the surface view. The absence of musculature prevents any independent movement of the fins. They appear to be only ectodermal expansions of the tail so that their structure is the same as the rest of the body-wall, except for the additional presence of fin rays and the amorphous basal substance. The fin is composed of the following parts: (1) epidermis; (2) tactile prominences; (3) a thin layer of basement membrane; (4) reticular parenchymatous tissue; (5) fin rays; (6) amorphous basal substance. Of these the first three have already been described, but it may be pointed out

that the layer of parenchymatous tissue is thick only in the basal region of the fins (Text-fig. 1, *p.t.*). Near the proximal end it is very thin and almost indistinguishable. The fin rays are embedded in the basal amorphous substance. They are very numerous and appear like thin strands originating from the distal end of the amorphous plate. In the lateral fins they are directed slightly backwards and run parallel to one another while in the tail fin they radiate in a fan-like manner from the posterior end of the tail. It seems probable that the rays are only local thickenings of the amorphous basal substance (Text-fig. 1 c, *amor.b.s.*) and that both together form a supporting skeletal structure which maintains the fin in the horizontal position. The amorphous basal substance is a structureless material which forms a median plate, staining deeply in haematoxylin. Its thick distal end is attached to the lateral-line between the dorsal and ventral bands of longitudinal muscles. The proximal end is very thin and it is difficult to fix its exact termination.

The Prehensile Spines¹ and Teeth.—In *Spadella cephaloptera* there are eight or nine prehensile spines of varying length, on each side of the head. Each spine consists of the following parts: a broad basal region, with a dorsal and ventral column for the attachment of the articulating muscles; a shaft, which has a sharp crest on the inner side; a 'point', with an oval base, which is embedded one-fourth to one-fifth of its height into the tip of the shaft; and a central pulp-cavity which extends to the middle of the 'point'. The pulp-cavity is only scantily filled with pulp.

The teeth are small conical structures with pointed tips. They, like the prehensile spines, are composed of a cuticular substance and vary in number from five to seven. The teeth are situated in a row, one each side round the anterior end of the lateral plate, and point downwards, but when the mouth is

¹ As the term 'Seizing Jaws' does not seem appropriate for the large hook-like setae, Prof. E. W. MacBride, F.R.S., suggested the name 'prehensile spines', and the new name is used throughout this paper and in the author's previous paper on the 'Anatomy of the head of *Sagitta*' (John, 1931).

opened they are turned towards the median line and serve to hold the prey while swallowing (fig. 18, *a.t.*, Pl. 36). In the normal condition the anterior end of the head is much contracted, so that the real position and function of the teeth cannot be clearly understood. The epidermis at the base of the teeth appears very thick and the teeth are situated in front of the mouth, but when the mouth is opened in the act of swallowing, it assumes an anterior terminal position and the teeth are then seen to fringe the sides of the lip. These alterations in the position of the teeth and mouth will be described in detail in the account of the muscular system.

The Lateral Plates.—The lateral plates, which constitute the chief skeletal structures of the head, are two in number and are placed dorso-laterally one on each side of the head. Each plate extends from the anterior end of the head to the posterior insertion of the *M. adductor uncinorum* (fig. 18, *l.p.*, Pl. 36). At the anterior end it is slender and rod-shaped, but it widens backwards and becomes flat and plate-like. It is composed of homogeneous non-staining cuticle and its ventral surface is attached to the underlying epidermal cells, which secrete the cuticular substance. The plates lie obliquely, so that the posterior ends diverge more towards the sides. The plate-like posterior end is slightly arched and roofs over the posterior region of the large *M. adductor uncinorum* (fig. 6, *l.p.*, Pl. 34). These plates give attachment to many of the muscles of the head, but their most important function is to open the mouth. This is a rather complicated process in which the muscles play an important part and so it is best to consider the function of the lateral plates in the account of the muscular system.

It has already been stated that the cuticular covering of the head is thicker than the rest of the body. On the ventral side of the head it forms two bands, one on each side, slightly thicker than the surrounding cuticle. These bands broaden posteriorly and extend up the sides. They have been called the ventral plates, but since they really form part of the cuticular envelope and are not differentiated into any definite structure, like the lateral plates, it does not seem essential to distinguish them

by any special name. These localized thickenings of the cuticle strengthen the ventral insertions of some of the anterior muscles of the head.

6. NERVOUS SYSTEM.

The nervous system of *Spadella cephaloptera* recalls the general disposition of the ganglia and nerves in other Chaetognatha. It consists chiefly of a brain and visceral ganglion. The brain gives off several nerves with accessory ganglia and is connected with the visceral ganglion in the trunk by a pair of commissures. The nerves given off from the brain supply the head and collar, and with those emerging from the visceral ganglion constitute the peripheral nervous system.

The brain is situated close to the anterior end of the head in the median dorsal line immediately below the epidermis. It is bounded on the sides by the *M. vestibuli internus* and ventrally by a thin plate of muscles, the *retractor preputii* (fig. 3, *m.r.pr.*, Pl. 34) which separates it from the general cavity of the head. A delicate layer of basement membrane envelops the brain completely except where the nerves pass through it. This basement membrane along with the adjacent muscle, *retractor preputii*, forms a closed capsule enclosing the brain. The *M. retractor preputii* is probably homologous with the *M. expansor subcerebrum* described by Grassi (1883) as forming the brain capsule. Gourret (1884) observed that the *M. expansor subcerebrum* is absent in *Spadella marioni*, but since he has not given any transverse sections passing through the brain it is not possible to say how far the homology is correct.

The size and position of the brain varies slightly in the different species. In *Sagitta bipunctata*, which is taken as a type owing to its abundance and constant occurrence, the brain is quadrangular in shape and lies half-way between the eyes and the anterior end. In *Spadella marioni* it is situated nearer to the eyes between the two *M. complex lateralis*, but in *Spadella cephaloptera* the brain lies close to the anterior end of the head and is oval in outline with the longest diameter placed at right angles to the long axis of the body.

The brain consists of a central fibrous mass, the 'punkt-substanz' (Text-fig. 3 A, *b.pnkt.*), which stains very lightly in haematoxylin and appears finely granular in sections. This is surrounded at the sides by an aggregation of ganglion cells. The distribution of the ganglion cells can best be seen in frontal and transverse sections of the brain. The lateral borders of the fibrous mass are surrounded by large clusters of ganglion cells (Text-fig. 3 A, *b.g.*). Round the anterior and posterior borders they are less numerous and fill only the spaces between the roots of the emerging nerves and commissures. A thin layer of nerve-cells occurs above the antero-dorsal border of the 'punktsubstanz'. The rest of the dorsal and ventral surfaces are free except for a few scattered cells.

Four pairs of nerves originate from the brain, the frontal and main commissures and the optic and coronal nerves. Of these, the first two are thick trunks, which spring from the anterior and antero-lateral borders, while the last two are slender and arise from the postero-lateral and posterior borders of the brain respectively.

The frontal commissure (Text-fig. 3 B, *f.c.*) originates from the antero-median end of the brain as a large nerve, which describes a sharp curve downwards and outwards in front of the M. expansus superior (fig. 1, *f.c.*, Pl. 34) and joins the vestibular ganglion. The vestibular ganglia are large and lie ventro-laterally on each side of the posterior half of the mouth. Their shape and position are shown in Text-fig. 3 B, *vest.g.* Close to the point where the frontal commissure joins the vestibular ganglion arises the frontal nerve (Text-fig. 3 B, *f.n.*), a small nerve, which runs towards the vestibular organ and the teeth. At the point of its origin it is slightly thicker, and this thickening has been regarded as a ganglion of the nerve, but since no ganglion cells are found around it, as in the case of other ganglia, it is doubtful whether this is a real ganglion.

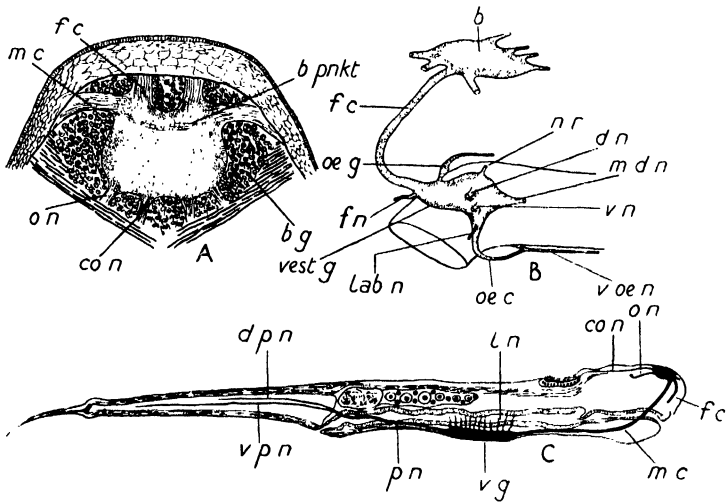
The oesophageal nerve arises from the inner side of the vestibular ganglion (fig. 2, *oe.g.*, Pl. 34). It runs dorsally and on reaching the roof of the oesophagus curves backwards in the wall of the latter. In *Sagitta bipunctata*, on the other hand, this nerve runs more towards the ventral side and is

embedded in the circular oesophageal muscles. There is a stout oesophageal ganglion close to the point of origin of this nerve. Beyond the ganglion the nerve becomes slender and it is very difficult to trace it in the longitudinal muscles along the sides of the oesophagus.

The position of the nerves given off from the vestibular ganglion is different in the different species, as will be best seen from a comparative account of these nerves in *Sagitta* and *Spadella*. In *Sagitta bipunctata* the dorsal nerve arises from the dorsal surface of the vestibular ganglion and bends backwards in the *M. expansus superior* beneath the lateral plate. The mandibular nerve originates slightly behind the dorsal, and the vestibular nerve arises more ventrally in almost the same transverse plane as the dorsal nerve. Posteriorly the ganglion gives rise to the oesophageal commissure, which embraces the ventral surface of the oesophagus and joins with its fellow from the opposite side. In *Spadella cephaloptera* the dorsal nerve arises dorso-laterally behind the *M. expansus superior* from the thickest part of the vestibular ganglion (fig. 3, Pl. 34, and Text-fig. 3 B, *d.n.*). It runs obliquely dorsally and backwards and enters the *M. adductor uncinorum* immediately below the lateral plate. The position of the vestibular nerve (*v.n.*) is the same as in *Sagitta bipunctata*; it runs downwards and outwards to the borders of the vestibule (fig. 3 B, *v.n.*, Pl. 34). A small nerve originates from the postero-dorsal surface of the ganglion and passes backwards close to the dorsal wall of the head. Though the root of the nerve is seen clearly in transverse sections the author was not able to trace it to its termination. It is probable that it supplies the *M. obliquus longus* and *M. retractor preputii* (Text-fig. 3 B, *n.r.*).

From the posterior end of the vestibular ganglion originates a thick nerve which probably corresponds to the mandibular nerve described by Burfield (1927) in *Sagitta bipunctata*. It runs backwards close to the inner edge of *M. complex lateralis* and enters the latter near its base (fig. 4, Pl. 34, and Text-fig. 3 B, *md.n.*). The oesophageal commissure originates from the postero-ventral region of the ganglion and runs downwards and inwards close to the posterior border of the mouth

(fig. 3, Pl. 34, and Text-fig. 3 B, *oe.c.*). On reaching the ventral surface of the oesophagus immediately behind the mouth, it bends backwards and joins with its fellow from the opposite side. At the junction of the two halves of the oesophageal commissure originates the slender unpaired ventral oesophageal nerve (Text-fig. 3 B, *v.oe.n.*), which runs backwards in the wall



TEXT-FIG. 3.

Nervous system. A. Brain. B. Cerebral ganglia and nerves. C. Main commissure and trunk-nerves. *b.* brain; *b.g.*, ganglion cells of the brain; *b.pnkt.*, punktsubstance of the brain; *c.n.*, coronal nerve; *d.n.*, dorsal nerve; *d.p.n.*, dorsal posterior nerve; *f.c.*, frontal commissure; *f.n.*, frontal nerve; *l.n.*, lateral nerve; *lab.n.*, labial nerve; *m.c.*, main commissure; *md.n.*, mandibular nerve; *n.r.*, nerve to *M. obliquus longus* and *M. retractor preputii*; *oe.c.*, oesophageal commissure; *oe.g.*, oesophageal ganglion and nerve; *o.n.*, optic nerve; *p.n.*, posterior nerve; *v.g.*, ventral ganglion; *vest.g.*, vestibular ganglion; *v.n.*, vestibular nerve; *v.oe.n.*, ventral oesophageal nerve; *v.p.n.*, ventral posterior nerve.

of the pharynx. This nerve, which is prominent in *Sagitta bipunctata*, is hardly distinguishable in *Spadella cephaloptera*. Close to its point of origin the oesophageal commissure gives off a small nerve called the labial nerve (Text-fig. 3 B, *lab.n.*).

The main commissures arise from the antero-lateral border

of the brain and pass outwards under the ectoderm at right angles to the long axis of the body (fig. 2, Pl. 34, and Text-fig. 3 A and c, *m.c.*). On reaching the sides they slowly bend backwards and run with a ventral inclination. The roots of the right and left nerves are connected across the brain by a transverse band of fibres. In the head the commissures lie beneath the basement membrane, but in the neck and trunk they are situated in the thick vacuolated layer of parenchymatous tissue close to the muscle-bundles. It has been suggested by Gourret (1884) that the main commissures pass backwards in the lateral line separating the dorsal and ventral bundles of longitudinal muscles. Neither in *Sagitta bipunctata* nor in *Spadella cephaloptera* is this found to be true. During their course backwards the commissures increase in thickness and lie close to the middle line of the ventro-lateral bundles of trunk muscles (fig. 7, *m.c.*, Pl. 34).

The optic nerves (Text-fig. 3 A, *o.n.*) spring from the postero-lateral borders of the brain. These nerves are very short; they pierce the basement membrane and enter the eyes anteriorly. The coronal nerves (*co.n.*) originate from the posterior border of the brain on each side of the median line. They run beneath the basement membrane and on reaching the corona ciliata break up into numerous branches which innervate this organ.

The ventral ganglion (*v.g.*) is elliptical in outline and is placed in the ventral wall of the trunk half-way between the neck and anal opening. In a full-grown specimen the ventral ganglion measures 0.5 mm. along the antero-posterior axis, while the brain measures only 0.05 mm. along the same axis. It is formed of a central 'punksubstanz', the lateral borders of which are surrounded by ganglion cells. Twelve nerves (*l.n.*) are given off from each side of the ganglion. These run upwards into the body-wall and divide into numerous branches. A pair of nerves called the posterior nerves (Text-fig. 3 c, *p.n.*) spring from the posterior end of the ventral ganglion. They diverge outwards and run backwards in the sides of the body-wall close to the ventro-lateral bundles of trunk-muscles. At the anterior end of the tail each nerve lies close to the lateral line between the dorso-lateral and ventro-lateral bundles of muscles. Here it

divides into two branches, the dorsal posterior and ventral posterior nerves of the tail (Text-fig. 3 c, *d.p.n.* and *v.p.n.*). These run in the dorsal and ventral angles on either side of the base of the amorphous substance of the fin (figs. 12-14, *d.p.n.* and *v.p.n.*, Pl. 35). The dorsal nerve is slender and ends in the middle of the tail, but the ventral nerve continues backwards to the region of the seminal vesicle before it becomes indistinguishable.

In Chaetognatha there is a network of minute nerve-fibres lying beneath the epidermis, called the peripheral nervous system. This forms a continuous plexus round the body, innervating the body-wall, the sensory pits, and probably the muscles of the trunk and tail. The plexus originates from the branches of the twelve pairs of trunk-nerves and from the posterior caudal nerves.

The Retrocerebral Organ.—In a typical case the retrocerebral organ consists of a pair of club-shaped bodies called the appendages, which are situated one on each side of the posterior region of the brain. These appendages are traversed by a narrow canal. The canals from the opposite sides converge towards the median line where they are connected with the single median external opening of the organ (John, 1931). In *Spadella cephaloptera*, though the median external opening, the two canals, and the appendages can be made out, the last two parts are very small and vestigial. The external opening is very prominent. It occurs as a vertical pit situated in the median line slightly behind the posterior border of the brain, and is bounded by large deeply-staining cells. The inner end of the external opening divides into two canals which run towards the right and left sides in the posterior region of the brain. At their extremities each canal is connected with a small cluster of cells which correspond to the appendages of the organ. But it has not been possible to discover whether the canals extend into the appendages as in *Sagitta elegans*.

7. MUSCULATURE.

The musculature of *Spadella cephaloptera* is exceedingly well developed and composed of powerful sets of muscles

with cross striated fibres. It resembles the muscular system of other Chaetognatha to a great extent in the general disposition of the various bundles, though some of the smaller muscles of the head with doubtful functions described in *Sagitta bipunctata* are not present in this species. The muscles are mainly divided into head and trunk muscles. There are no separate caudal muscles, as the longitudinal muscles of the trunk continue uninterrupted into the tail. The trunk muscles are composed of simple longitudinal bundles, while the cephalic muscles show a complex arrangement.

The head musculature first described rather imperfectly by Hertwig (1880) was made the subject of special research by Grassi (1883) who named the different muscles and attempted to explain their functions. In *Sagitta bipunctata* there are fifteen paired muscles and three unpaired muscles in the head, while in *Spadella cephaloptera* there are only eleven paired and three unpaired muscles which are grouped as follows:

Paired Muscles.

1. *Expansus superior*.
2. *Constrictor oris primus*.
3. *Dilator vestibuli externus*.
4. *Adductor uncinorum*.
5. *Dilator vestibuli internus*.
6. *Complex lateralis*.
7. *Obliquus longus*.
8. *Obliquus superficialis*.
9. *Circular oesophageal*.
10. *Transverse dorsalis*.
11. *Retractor preputii*.

Unpaired Muscles.

1. *M. bicornis*.
2. *Transverse ventralis*.
3. *Protractor preputii*.

Since some of the larger muscles are rather opaque and overlap the smaller ones lying deep in the head, the muscular system

could not be successfully studied in living specimens or whole mounts, and so the following account is based on transverse, frontal, and sagittal sections of the head. Each muscle is described in the order given below.

1. *M. expansus superior* (fig. 1, *m.e.s.*, Pl. 34) is the most anterior muscle in the head lying dorsal to the anterior end of the mouth opening, behind the curve of the frontal commissure. It is composed of transverse fibres, the inner ends of which are attached to the median connective tissue lamella. The outer ends are inserted on the inner side of the anterior end of the lateral plate, and the bases of the anterior teeth. It is probable that the fibres passing into the anterior teeth may correspond to the *M. obliquus capitis brevis*, but as they are not separated into different bundles such a distinction does not seem necessary.

2. *M. constrictor oris primus*.—In *Sagitta bipunctata* the *M. constrictor oris primus* and *M. constrictor oris* act together and serve to close the mouth and vestibule, but in *Spadella cephaloptera* the latter muscle is absent. *M. constrictor oris primus* is a band-like muscle with oblique fibres. Its anterior insertion is on the median connective tissue lamella, below the insertion of *M. expansus superior* (fig. 2, *m.c.o.p.*, Pl. 34). From here the fibres pass downwards and backwards and their ends are attached to the middle of the lateral border of the mouth in front of the ventral insertion of *M. dilator vestibuli internus* (fig. 4, *m.c.o.p.*, Pl. 34).

3. *M. dilator vestibuli externus*.—This muscle occurs immediately behind the *M. expansus superior* between the lateral ectoderm and the vestibular ganglion (figs. 2, 3, and 4, *m.d.v.e.*, Pl. 34). It is composed of transverse fibres which run in a dorso-ventral direction, the dorsal insertion being slightly behind the ventral. Dorsally the fibres are attached to the outer side of the lateral plate and the ventral insertion is in the ectoderm forming the lateral border of the vestibule.

4. *M. adductor uncinorum*.—This is a pair of large muscles situated in the dorso-lateral surface of the head, and extending from the posterior border of the lateral plate (fig. 6, *m.ad.*, Pl. 34) to the region of the dorsal insertion of *M. dilator*

vestibuli internus (fig. 6, *m.ad.*, Pl. 34). The hinder half of the muscle is very thick and broad, but it tapers towards the anterior end. The fibres run in an oblique longitudinal direction from their origin on the inner border of the flat posterior end of the lateral plate. The outer ends are inserted on the ventral columns of the prehensile spines and the lateral wall of the head in front of the prehensile spines.

5. *M. dilator vestibuli internus*.—This muscle occurs immediately behind the vestibular ganglion between the anterior end of *M. complex lateralis* and the pharynx. It is composed of transverse fibres running in an oblique dorso-ventral direction. The ventral insertions are on the inner side of the vestibule close to the posterior border of the mouth (fig. 3, *m.d.v.i.*, Pl. 34). The function of this muscle has not been properly studied in *Sagitta*. It is believed that in a general way it serves to open the mouth. It will be seen later that in *Spadella*, the contraction of this muscle draws the posterior border of the mouth inwards when the mouth closes. The name of this muscle, therefore, is misleading at least in the case of *Spadella cephaloptera*. It would be more appropriate to call it the muscle retractor oris, but as such an alteration may bring about some confusion in the nomenclature, the older name is retained in this paper.

6. *M. complex lateralis*.—This is a pair of large muscles lying beneath *M. adductor uncinorum* and extending from the posterior end of the head to the sides of the *M. bicornis*. The hinder region of this muscle is very thick and broad and occupies the greater part of the sides of the head (fig. 5, *m.c.l.*, Pl. 34). It is composed of many bundles, the fibres of which are obliquely transverse. At the anterior end it is inserted on the sides of the median unpaired *M. bicornis*, and the posterior attachment is on the outer side of the hind end of the lateral plate. Some of the fibres pass into the sides of the head on the ventral face of the lateral grooves. These grooves were not described in the account of the external characters of the animal as they are not permanent structures of the head. When the prehensile spines are closed they lie in a shallow longitudinal groove on each side of the head, but when the spines are opened

the grooves are completely evaginated. Some of the fibres of both *M. adductor uncinorum* and *M. complex lateralis* are attached to the ectoderm forming these grooves.

7. *M. obliquus longus*.—This is a slender bundle of longitudinal fibres situated close to the median line in the dorsal part of the head. From its anterior insertion on the anterior end of the lateral plate (fig. 1, *m.o.l.*, Pl. 34) it runs backwards, and the posterior end is attached to the dorsal wall of the head in front of the eyes (fig. 5, *m.o.l.*, Pl. 34). Behind the region of the mouth it runs close to the outer side of *M. obliquus superficialis*.

8. *M. obliquus superficialis*.—This muscle, composed of longitudinal fibres, is situated in the dorsal part of the head. The two bundles of the pair run close together, being separated only by a median vertical connective tissue lamella. The posterior ends of these muscles are very narrow and are wedged in between the two dorsal bands of longitudinal trunk muscles, above the intestinal diverticula (fig. 7, *m.o.s.*, Pl. 34). The bundles become wider and thicker at about a third of their distance from the posterior end (fig. 6, *m.o.s.*, Pl. 34), and then slowly narrow down again towards the anterior end. The anterior insertion is on the dorsal wall of the head in the region of the *M. bicornis* (fig. 5, *m.o.s.*, Pl. 34).

9. *M. circularis oesophagi*.—This muscle is described in the account of the alimentary system.

10. *M. transversus dorsalis*.—This pair of muscles is situated in the posterior dorsal part of the head. From their insertion on the median connective tissue lamella the fibres run outwards, and are attached to the inner posterior end of the lateral plate and *M. adductor uncinorum* (fig. 6, *m.t.d.*, Pl. 34).

11. *M. retractor preputii*.—This is a small pair of transverse muscles situated in the anterior end of the head beneath the brain (fig. 3, *m.r.pr.*, Pl. 34). Their median insertions are on the thick plate-like connective tissue forming the floor of the brain and their outer insertions are on the dorso-lateral wall of the head.

12. *M. bicornis*.—This is a thick unpaired sausage-shaped muscle lying in the ventral part of the head, behind the

posterior border of the mouth, with its long axis situated at right angles to the long axis of the body (fig. 5, *m.b.*, Pl. 34). It is concave in front and convex behind, and it is wrapped round with a thin structureless membrane. Its fibres run in an oblique dorso-ventral direction, in such a way that its dorsal ends are attached more laterally than the ventral. It has already been stated that the anterior ends of some of the fibres of the *M. complex lateralis* are attached to the ends of this muscle. The intramuscular organ which is found embedded in the centre of this muscle in most of the species of *Sagitta* is absent in *Spadella cephaloptera*.

13. *M. transversus ventralis*.—This is an unpaired muscle lying on the ventral part of the neck beneath the anterior ends of the longitudinal trunk muscles and the oesophagus (fig. 6, *m.t.v.*, Pl. 34). The fibres run transversely and their ends extend into the ventral part of the hood.

14. *M. protractor preputii*.—This is a thin unpaired circular muscle extending round the anterior margin of the hood. This is the only muscle in the hood, and it acts as a sphincter for regulating the size of the hood opening. When the hood is drawn over the head its opening is very narrow and is situated antero-ventrally surrounding the vestibule, but when the hood is pulled backwards the sphincters relax and the opening surrounds the posterior half of the head (figs. 18 and 19, *h.*, Pl. 36).

Functions of the Cephalic Muscles.—In attempting to discover the functions of the various muscles of the head previous workers based their inferences on sections and whole mounts of specimens in which the mouth and prehensile spines were closed. In this condition the mouth is placed antero-ventrally and the hood is drawn over the anterior end of the head. When the mouth was opened in the act of catching prey the process was believed to be only a widening of the oval aperture of the mouth and the vestibule. The vestibule is a shallow depression surrounding the mouth, and it was supposed that the widening of this depression was correlated with the opening of the mouth. Though this was regarded as the correct mode of food capture in *Sagitta*, the process in *Spadella* is somewhat different.

In *Spadella cephaloptera* the mouth occupies an antero-ventral position when closed (fig. 19, Pl. 36), but assumes an anterior terminal and vertical position when opened. The hood is drawn backwards and the dorso-lateral edges of the mouth are supported by the anterior ends of the lateral plates. The teeth are situated on the margin of the mouth and the curved pointed ends are directed towards the opening (fig. 18, Pl. 36). When the mouth is closed it is drawn inwards and ventralwards. The vestibule is a temporary depression which is formed only when the mouth is closed. It is completely evaginated when the mouth opens.

The functions of the various cephalic muscles in *Spadella cephaloptera* have therefore to be interpreted in the light of these new facts. It is to be noted that the length of the head is not increased when the mouth opens, so that the new position of the mouth opening is brought about only by the adjustment of some of the head muscles.

Before the mouth opens the hood is drawn backwards (figs. 17 and 18, *h.*, Pl. 36). This is brought about by the contraction of the *M. obliquus superficialis*. This process also lifts the head slightly upwards, while a more vigorous contraction of this muscle, in conjunction with the dorsal longitudinal muscles of the trunk, enables the animal to bend its head backwards and pick up with its mouth anything adhering to the aperture of the female genital opening; for instance, during sperm transference, if the two individuals are disturbed, they swim apart and the mass of spermatozoa adhering to the vaginal opening is picked up and swallowed. When the hood is drawn backwards the *M. protractor preputii* relaxes, increasing the circumference of the hood opening, and thus creating more space between the hood and the sides of the head for the movement of the prehensile spines.

The shape of the head is slightly altered when the hood is withdrawn owing to the contraction of *M. bicornis*. The anterior ends of some of the fibres of *M. complex lateralis* are attached to the sides of *M. bicornis*, so that when the former muscle contracts it pulls the sides of the *M. bicornis* slightly backwards, thus making it longer and straighter (fig. 17, *m.b.*,

Pl. 36). The backward pull of *M. obliquus superficialis*, *M. complex lateralis*, and *M. adductor uncinorum*, indirectly causes the anterior ends of the lateral plates to project forward. At the same time the *M. constrictor oris primus* contracts and pulls the mouth anteriorwards. Thus, by the combined action of all these muscles, the mouth becomes an anterior vertical slit (fig. 17, Pl. 36). In this condition no trace of the vestibule can be observed, and unlike *Sagitta* it can be demonstrated that the vestibule does not play any part in food capture or in the function of the mouth. When the chief muscles of the head contract, and the hood is withdrawn, the vestibule is completely evaginated and the mouth is seen projecting anteriorly as a short transparent tube, supported at the sides by the anterior ends of the lateral plates. In the normal state the lateral plates are not seen except in transverse sections. They lie obliquely on the dorsal surface overlapping the large muscle-bundles on each side, but when the hood is withdrawn the anterior rod-shaped parts of the lateral plates project about a third of their length in front of the chief muscle-bundles. This is not brought about by the protrusion of the anterior ends of the head but, as already stated, by the contraction of *M. complex lateralis* and *M. adductor uncinorum*. This is shown by the fact that the length of the head from the corona ciliata to the anterior end is not increased when the mouth assumes its anterior terminal position.

The *M. dilator vestibuli externus* is attached to the outer side of the anterior end of the lateral plate, and its insertion is in the ectoderm forming the lateral border of the vestibule in the normal condition, but when the mouth assumes the terminal position this muscle becomes vertically transverse, bounding the lateral wall of the oesophagus below the lateral plates. This muscle, together with the *M. expansus superior*, functions like a sphincter for regulating the orifice of the mouth during food capture. The opening and closing of the oral aperture is controlled by the anterior ends of the lateral plates in conjunction with *M. expansus superior*, *M. obliquus longus*, and *M. transversus dorsalis*. The *M. transversus dorsalis* is attached to the inner posterior end of the lateral plate, and when it contracts it draws the posterior end of the lateral plate close to the

median line, thus causing the anterior ends to diverge. Since the anterior ends are attached to the lateral edges of the mouth, this divergence tends to open the oral aperture. The closure of the oral aperture is brought about by the contraction of *M. obliquus longus* and *M. expansus superior*.

From this account it will be noticed that the lateral plates function like jaws. They support the mouth and act as mechanical levers for opening and closing the oral aperture. In *Sagitta*, on the other hand, it has been suggested that the chief function of the lateral plates is to give attachment to some of the cephalic muscles, and by its movement to help indirectly to open the prehensile spines. Though these functions are not ignored in the present account, it seems more than probable that their real function has not been properly understood chiefly because of the fact that in *Sagitta* it is not definitely known what position the mouth assumes during food capture.

The alteration in the shape of the head is the chief factor which shifts the mouth and the ends of the lateral plates forwards. During these alterations the *M. bicornis* seems to play a very important part. In the normal condition this is a transversely placed sausage-shaped bundle of unpaired muscle-fibres, but when the mouth is shifted forward it becomes considerably elongated and its fibres are directed more transversely. The exact significance of this change in relation to the opening of the mouth is not clearly understood, but it is believed that it functions in co-ordination with the *M. complex lateralis* as already explained.

The articulation of the prehensile spines is controlled by the *M. adductor uncinorum* as in *Sagitta*. The anterior teeth change their positions when the mouth shifts forward, and come to lie on the lateral edge of the mouth with their curved pointed ends directed transversely inwards. They serve to give a firm hold on the prey when it is being swallowed, and are worked by some of the fibres of the *M. expansus superior*.

After food has been swallowed the hood comes back to its original position aided probably by the contraction of *M. obliquus longus*. The contraction of this muscle along with *M. expansus superior* also brings the anterior ends of the lateral

plates closer together and thus closes the oral aperture; at the same time the *M. dilator vestibuli internus* contracts and draws the posterior ends of the mouth ventralwards to its original position (fig. 19, Pl. 36). It is probable that the vestibule is formed by the contraction of this muscle.

The Musculature of the Body.—The muscles of the trunk and tail are arranged in a comparatively simple manner. There are four bundles of longitudinal muscle-fibres extending from the hind end of the head to the tip of the tail, and two oblique transverse bands confined only to the trunk. The longitudinal muscles are disposed in a pair of dorsal and a pair of ventral bands, separated laterally by a row of epithelial cells called the lateral line (fig. 14, *l.l.*, Pl. 35). The two bundles of the dorsal and ventral pairs are separated from each other by a thin median septum, so that the four bundles are quite distinct in transverse sections.

The nature and position of the longitudinal bands are slightly different behind the posterior end of *M. complex lateralis*, where it occurs as a tubular process on each side of the oesophagus, enclosing the anterior end of the trunk coelom (fig. 6, *v.l.m.* and *d.l.m.*, Pl. 34). These tubular extensions are situated more towards the ventral part of the head, and each is bounded by a single dorsal and ventral longitudinal bundle. As they proceed backwards the trunk cavities slowly widen and completely surround the oesophagus. The two dorsal bands now approach each other, but are still separated by the intervention of the posterior ends of the *M. obliquus superficialis*, which, as has already been stated, is wedged between the anterior ends of the right and left longitudinal muscles of the trunk (fig. 7, *d.l.m.* and *m.o.s.*, Pl. 34). In this region the two ventral bands are close together in the ventral median line, being only separated by the ventral median septum. Near the anterior ends of the ovaries the two ventral bands become slightly thinner and broader than the dorsal bands, and separate from each other along the ventral median line (fig. 9, *v.l.m.*, Pl. 35). This separation is more marked in the region of the visceral ganglion. The visceral ganglion is covered internally by a layer of epithelial cells, and the ends of the ventral bands appear to be

attached to the sides of this layer. Near the posterior end of the alimentary canal the ventral bundles become ventro-lateral, and the median ventral body-wall is very thin (figs. 9 and 10, *v.l.m.*, Pl. 35). In the region of the seminal receptacles (fig. 11, *sem.rec.*, Pl. 35) the dorsal and ventral bundles separate along the lateral lines, giving room for the vaginal openings. In the tail the bundles again approach each other, and are only separated by the median septum and the lateral line of epithelial cells (fig. 16, *d.l.m.* and *v.l.m.*, Pl. 35).

In the trunk there is an oblique band of transverse muscle-fibres on each side of the alimentary canal (figs. 9 and 10, *o.t.m.*, Pl. 35). The dorsal ends of the fibres are inserted in the middle of the row of epithelial cells separating the dorsal and ventral bundles of longitudinal muscles, and their ventral ends are attached to the ventro-median sides of the ventral longitudinal muscles. These thin bands of oblique muscles, therefore, correspond in position to the oblique muscles in Archiannelids and Polychaets. The longitudinal muscle-bundles are used in swimming and darting movements of the animal, and the fibres of these muscles are arranged on the type found in earthworms. In transverse sections they appear to consist of a number of lamellae, placed like the barbs of a feather, overlapping one another and inclined at an angle of 45° with the outer surface of the body.

8. COELOM.

The coelomic cavities appear early in development and are primarily three in number, a head cavity, and a pair of trunk cavities. In the adult the trunk cavities are secondarily divided into trunk and tail cavities by the development of a transverse septum.

The coelom in the head is very much reduced owing to the great development of the cephalic muscles. It can be seen in transverse sections, traversed by the various muscles. There does not appear to be any distinct coelomic epithelium, unless the delicate covering of some of the muscles is regarded as such. In transverse sections the cavity is seen to surround the oesophagus. It is wide on the dorsal side, but laterally and ventrally

it is very much compressed owing to the great development of *M. complex lateralis* and *M. bicornis*. The wide dorsal part of the coelom extends into the hood.

The trunk cavities envelop the alimentary canal which is kept in position by the dorsal and ventral median longitudinal mesenteries. Anteriorly the trunk cavities separate into right and left tubular horns which extend to the posterior end of the *M. complex lateralis*. In the trunk there are two bands of oblique muscle-fibres, one on each side of the alimentary canal. They run from the lateral line, obliquely ventralwards, and are inserted in the ventro-median edges of the ventral longitudinal muscles. These oblique muscle-fibres traverse the trunk cavity, but in the posterior region of the trunk, owing to the great development of the ovaries, they are pressed close to the inner surface of the ventral bands of longitudinal muscles.

Behind the termination of the alimentary canal the median mesenteries are at first divergent and enclose a tunnel between them which extends a short distance into the tail (figs. 13 and 14, s.s., Pl. 35). This was regarded as the posterior extension of the trunk cavities, but it will be seen, from fig. 13, *coel.t.*, Pl. 35, that the posterior end of the trunk cavities form narrow canals close to the lateral body-wall. The median tunnel is not part of the coelom. It has been shown by Meek (1928) that this tunnel represents the space occupied by the intestine, which in the primitive Chaetognatha, *Amiskwia sagittiformis* (Walcott, 1911), extended to near the posterior end of the tail. In the living representatives the alimentary canal has been reduced considerably in length, and the tunnel between the septa represents a part of the primary body-cavity which, in the primitive condition, was occupied by the posterior extension of the alimentary canal. Behind the tunnel the two mesenteries come together and form the longitudinal median vertical mesentery which extends to the tip of the tail. This median septum divides the tail coelom into a right and left chamber. In addition, it has been stated that in *Sagitta* there is a secondary longitudinal septum in each chamber lying parallel to the median septum, but in *Spadella cephaloptera* no such partition is visible.

9. ALIMENTARY SYSTEM.

The alimentary system consists of the mouth, oesophagus, intestine, and anus. The working of the mouth in relation to the vestibule or buccal cavity has already been described in the account of the muscular system. The vestibule is only a temporary depression at the bottom of which lies the mouth in the normal condition. It is completely evaginated when the mouth assumes its anterior terminal position.

When the mouth is opened the alimentary canal becomes a straight horizontal tube, thus enabling any large prey to be swallowed without difficulty. The lateral free edge of the mouth bears the small conical teeth, and the sides are supported by the anterior rod-shaped ends of the lateral plates, which function like jaws for opening and closing the oral aperture. On closure the mouth resumes its antero-ventral position and is surrounded by the free edges of the hood. The prehensile spines are important organs for catching and holding prey, and probably serve for defence also.

The mouth is followed by the oesophagus which extends the entire length of the head to the region of the neck. It has the form of a narrow tube expanding into a bulb-like swelling at its posterior end. In transverse sections the dorsal half of the oesophageal wall is thick, while the ventral half is rather thin and has the appearance of an inverted flask, the swollen part being represented by the thick-walled dorsal region (fig. 5, Pl. 34).

The size and nature of the epithelial cells vary in different parts of the oesophagus. In the dorsal half the cells are large and cylindrical, with basal nuclei, but in the ventral half the cells are very much smaller. The outer ends of the cells contain aggregations of granules which stain very deep in haematoxylin. The dorsal cylindrical cells are highly glandular, but it is doubtful whether the smaller cells have any glandular function. Beneath the epithelial layer is a thin layer of basement membrane which appears like a well-defined line in transverse sections. This is surrounded by a layer of circular muscles which extends the entire length of the oesophagus. At the

posterior end of the mouth this muscle is attached dorsally to the covering layer of *M. protractor preputii*, and ventrally it is inserted on the ventral ectoderm below the vestibular ganglion. Behind the mouth the median ventral insertion shifts to the median constriction of the *M. bicornis*. From the commencement of the *M. obliquus superficialis* the circular muscle is attached dorsally to the sides of the former; still further behind it is attached to the median dorsal mesentery and to *M. transversus ventralis*.

Along the mid-lateral line on each side, in the region of the junction of the dorsal and ventral halves of the oesophagus, there is a thin band of longitudinal muscles between the basement membrane and the circular muscle. In *Sagitta bipunctata* this is said to form a continuous layer round the oesophagus, but even the minutest examination does not show any trace of such a continuous layer. The only longitudinal muscle-fibres in the oesophageal wall are those described above. They are very small bands which become weak anteriorly and posteriorly, so that it is difficult to fix their terminations.

The intestine is a straight tube which extends from the neck region to the anus. At the point where the oesophagus opens into the intestine, there is on each side a short stumpy diverticulum of the intestine. These diverticula are directed obliquely forward (fig. 7, *int.div.*, Pl. 34), and the lining epithelial cells are short and thick with centrally placed nuclei and lightly-staining protoplasm. The shape and structure of the intestine varies in different regions. At the beginning it is circular in cross section and the internal surface is thrown into ten or twelve ridges. In the region of the ovaries it lies close to the ventral body-wall and is slightly compressed from side to side in cases where the ova are well developed. Near the anus the ridges almost disappear and the wall is comparatively thin. The cavity inside is more spacious and it opens externally by the anus. This last part, since it is different in structure from the rest of the intestine, may be distinguished as the rectum.

The epithelial layer of the intestine consists of three kinds of cells: cylindrical granular cells, cylindrical non-granular cells, and short intermediate cells. The ridges in the anterior region

of the intestine (fig. 8, *int.*, Pl. 34) consist of groups of four or five cylindrical cells which project into the lumen. In transverse sections each ridge consists of a few granular cells alternating with non-granulated cells, though the alternation may not be very regular in all cases. The short intermediate cells occur between the ridges, and they resemble the cylindrical cells in being either granular or non-granular. In all the epithelial cells the nucleus is situated basally. In the granular cells the granules are packed in the outer ends, as in the cells lining the oesophagus, and like the latter their function is secretory. The other type of cells are slightly narrower and they are called absorbent cells.

Towards the posterior end of the intestine the ridges become smaller and are confined only to the ventral half of the intestinal wall (fig. 11, *int.*, Pl. 35). In the rectum the epithelial cells are short and undifferentiated and appear to be ciliated.

The oesophageal diverticulum and vestibular organs are absent in *Spadella cephaloptera*. In the region of the vestibular pit there appears a thickening of the ectoderm, consisting of large glandular cells, which is probably comparable to the pit itself.

10. REPRODUCTIVE ORGANS.

Spadella cephaloptera is hermaphrodite. The female organs occur in the posterior region of the trunk and the male organs in the tail. The male reproductive organs consist of a pair of testes, vasa deferentia, and sperm vesicles. The testes occur one in each chamber at the anterior end of the tail. In a young *Spadella* the testis is a small spherical organ, attached to the inner surface of the dorsal longitudinal muscles (Text-fig. 5 B). Later it develops into a horizontal band-shaped structure which extends to the posterior end of the lateral fin. It is covered by a thin epithelium, between which and the body-wall there is considerable space filled with clear coelomic fluid. As the testis matures it buds off masses of sperm mother-cells into the coelomic fluid. This takes place so profusely that in a mature specimen the cavity is completely crowded with these floating masses which maintain a constant circular movement as in *Sagitta*. In each chamber these sperm masses move

forward close to the outer side, and on reaching the anterior end curve backwards close to the median septum. In *Spadella* this movement is very slow and it takes four or five minutes to complete one circle. Bordas (1912) suggested that the circular movement is caused by the actively moving ripe spermatozoa. As this did not fully account for the regular circulatory movement, Burfield (1927) concluded that it is effected by the action of cilia, which he believed to be present on the surface of the median longitudinal septum. Against the latter opinion it has to be said that no trace of the presence of cilia has been observed on the median septum either in *Sagitta* or *Spadella*.

The circulatory movement is noticeable only in specimens with ripe spermatozoa, and even in them certain masses are seen to move more vigorously than others. These moving active masses are probably masses of ripe spermatozoa, which move about by lashing their tails, and not by any ciliary action as supposed by Burfield (1927). This leaves the problem of the regular circulatory movement unexplained. It will be noticed that such movement is essential for the passage of the ripe spermatozoa into the internal opening of the vas deferens. In a mature specimen each chamber of the coelomic cavity is crowded with the floating masses of sperm cells which are in different stages of maturation. The tails of the ripe masses lash in such a way that these masses always move forward in one definite direction, and since the central median part is occupied by the testis, free space is obtained only at the sides close to the body-wall and the septum. Masses of ripe spermatozoa, found close to the outer body-wall, move forward, because, as already explained, the tails lash only in one definite direction. When they reach the anterior end their further progress is checked by the curved transverse septum, so they turn round towards the median septum, and the tails still working in the same direction as before propel them towards the posterior end, thus setting up a slow circulatory movement. This explains why such a movement is seen only in mature specimens. Ripe masses of spermatozoa alone can propel themselves, and they do so in their endeavour to reach the opening of the vas deferens.

Vasa deferentia.—These ducts are found one on each side near the posterior end of the tail. Each duct is a short narrow tube running parallel to the long axis of the body. It opens internally into the tail cavity through the genital funnel (coelomostome). This internal opening is situated in the lateral line, slightly dorsally at the lower edge of the dorsal longitudinal muscles (Text-fig. 4 c, *int.op.*). The lips of the genital funnel are formed of deeply-staining columnar cells which appear to be ciliated at least around the internal opening of the duct, though the cilia are not quite visible in sections. The columnar cells of the funnel are formed from the lateral coelomic epithelium between the dorsal and ventral bands of longitudinal muscles, but are distinguished from the latter by the presence of large deeply-staining nuclei. The anterior lip extends nearly two-thirds the length of the tail, but the posterior lip is comparatively short (Text-fig. 4 D, *f.m.d.*). From its internal opening the duct runs backwards. During the first half of its course it is pressed close to the outer surface of the posterior lip of the genital funnel, and is situated dorso-laterally above the amorphous basal substance of the lateral fin (Text-fig. 4 B and D, *m.d.*). At the posterior end of the lateral fin the amorphous basal substance disappears, and at this point the duct bends slightly downwards and outwards and occupies a mid-lateral position (Text-fig. 4 A, *m.d.*). At its posterior end the duct widens slightly and opens into the anterior end of the sperm vesicle.

Sperm vesicles.—The sperm vesicles are situated laterally, one on each side of the posterior end of the tail, wedged between the lateral and caudal fin. Each is placed inside the body-wall and does not project out as in *Sagitta*. Each vesicle is oval in shape, with its long axis parallel to the long axis of the body. The vas deferens opens at the anterior end by a narrow funnel-shaped opening, and the external opening of the vesicle is situated at its posterior end. The vesicle is covered on the outer side by a thick stratified epidermis and its inner side rests against the lateral line between the muscular bundles (fig. 16, *ves.sem.*, Pl. 35). It is lined internally by an epithelium of short cubical cells which do not appear to be glandular, as it is stated

that they are in *Sagitta*. In the adult the sperm vesicles are filled with filliform spermatozoa.

Female Reproductive Organs.—The female reproductive organs consist of an ovary, oviduct, seminal receptacle, vagina, and cement gland on each side. The ovaries are situated in the posterior end of the trunk cavities and are separated from each other by the median septum and the intestine. Each ovary is an elongated cylindrical body covered with a thin layer of epithelium. In a mature specimen it consists of six to eight large eggs and numerous smaller ones in different stages of development. The ovaries extend nearly two-thirds the length of the trunk and are placed near to the dorsal surface. In this region the alimentary canal sinks towards the ventral surface and touches the mid-ventral body-wall. The body is slightly enlarged and the greater part of the cavities is occupied by the two ovaries. The oblique muscular fibres which are clearly discernible in front of the ovaries are now pressed to the inner surface of the ventro-lateral longitudinal muscle. Fully developed eggs are very large and fit closely inside the ovaries. They are arranged in linear order, and between them at the sides a few smaller eggs may be found. The smallest and youngest oocytes occur in a solid cluster at the very anterior end of the ovary (Text-fig. 4 E and F). At its posterior end the ovary opens directly into the dorsal part of the seminal receptacle through a short oviduct (Text-fig. 4 E, *sem.rec.o.*).

The seminal receptacle is a large slightly bilobed pouch usually filled with masses of active spermatozoa. At the level of the rectum it is very large in transverse sections and fills the greater part of the trunk cavity (fig. 11, *sem.rec.*, Pl. 35). Anteriorly it is partially divided into two tubular extensions placed one above the other. The ovary opens into the dorsal part (Text-fig. 4 E, *sem.rec.o.*), whilst the ventral part is drawn out into a narrow tube which runs the entire length of the ovary on its ventral side (Text-fig. 4 E, *s.d.*). Near its commencement a lumen is recognizable, but farther forward the tube collapses, so that in transverse sections it is seen only as two layers of cells.

There are certain very essential differences between the female reproductive organs in *Sagitta* and *Spadella*,¹ and to make these clear it is necessary to give a comparative account of both types. In *Sagitta* (Stevens, 1910) there is a double duct, one inside the other, extending along the outer side of each ovary and opening posteriorly at the level of the rectum into a small bulb-like seminal receptacle. The inner tube of the duct is called the 'samentasche' (sperm pouch). Surrounding this, and fitting closely over it, is an outer duct called the oviduct. Both these ducts end blindly at the anterior end of the ovary, whilst posteriorly they open by separate openings into the seminal receptacle. When an egg is fully developed the nuclear membrane disappears and the egg begins to push its way actively through the wall of the oviduct, at any point along its length, into the space between the sperm pouch and the oviduct wall. This it does by its own contractions and by shifting of material within the egg membrane. Through this duct the egg moves backwards and on reaching the lower end passes out through the external opening. In *Spadella cephaloptera*, on the other hand, the ovary opens directly into the dorsal part of the seminal receptacle. On the ventral surface of the ovary there is a narrow tube which forms a continuation of the ventral part of the seminal receptacle. In most cases the lumen inside this tube is not visible, so that it has the appearance of a crescent-shaped double layer of cells attached to the ventral surface of the ovary. This is not a double tube as in *Sagitta*, and the youngest oocytes are not found within the median central part of the crescent, but at the anterior end of the ovary. Since the ovary opens directly into the seminal receptacle, this tube can only be compared to the 'samentasche' (i.e. the inner tube of the duct of *Sagitta*), but it is to be noted that no

¹ The account of Vasiljev, 1925, of the female reproductive organ of *Spadella cephaloptera* suggests a close similarity in the structure of this organ with that of *Sagitta*. This is obviously due to the fact that the direct opening of the ovary into the seminal receptacle is visible only when the eggs are passing out. The present account is based on specimens which were kept under observation and fixed at the time when the eggs were being extruded. In sections made from this the opening of the oviduct is very large and unmistakable.

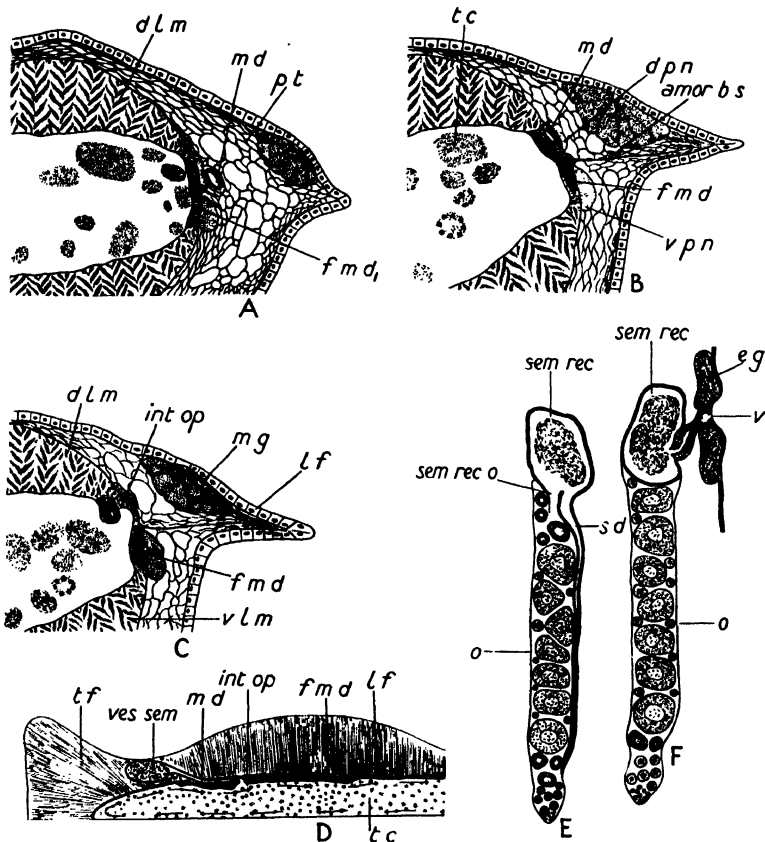
trace of spermatozoa has been observed inside it in any of the specimens so far examined.

From the mid-lateral region of the outer side of the seminal receptacle a short tube leads outwards and opens externally at the level of the rectum (fig. 11, v., Pl. 35). In the present paper this has been termed the vagina. It is a thick-walled ciliated tube running in an oblique transverse plane. Its internal opening is situated at about one-third the distance from the posterior end of the seminal receptacle (Text-fig. 4 F, v.), and it opens externally into a shallow epidermal pit of the lateral body-wall. This pit is lined by the cells of the cement gland (Text-fig. 1 A) which has been described already.

The eggs when fully developed are very probably fertilized by spermatozoa entering the ovary through the samentasche, but since no communication between this duct and the ovary has been observed, the only possibility seems to be that the spermatozoa enter the ovary in the same way as the eggs pass into the oviduct in *Sagitta*, by pushing their way between the cells lining the duct. The fertilized eggs move backwards through the oviduct into the seminal receptacle and enter the vagina. On reaching the external opening of the vagina the secretion of the cement gland forms a thin covering round the egg with a stalk on one side. Six to eight eggs are thus discharged at the same time from each ovary. They are held together in a cluster by their stalks and attached to some foreign object on which the animal rests at the time of egg laying.

11. REPRODUCTION.

Spadella cephaloptera lays about twelve to sixteen eggs at intervals of eight to ten days all the year round. In captivity the number of eggs is reduced and the interval slightly more prolonged. If food is abundant and the water of the containing vessel kept pure, captivity does not produce any retarding influence on breeding. Accordingly, in order to obtain optimum conditions, each individual was separately fed with small copepods three or four times a day and the water kept constantly renewed by a simple process of capillary action which avoided all disturbances in the containing vessel.



TEXT-FIG. 4.

Figs. A, B, and C. Sections passing through different regions of the male duct (drawn with the aid of camera lucida). Fig. D. Diagram of the right half of the tail region showing the structure and relation of the genital funnel with the internal opening of the duct. (The arrows mark the direction of circulatory movement of the sperm mother-cells.) Figs. E and F. Sagittal and frontal view of the female reproductive organ. *amor.b.s.*, amorphous basal substance; *c.g.*, cement gland; *d.l.m.*, dorsal longitudinal muscles; *d.p.m.*, dorsal posterior nerve; *f.m.d.*, genital funnel; *f.m.d.*, posterior end of the genital funnel; *int.op.*, internal opening of the male duct; *l.f.*, lateral fin; *m.d.*, male duct; *m.g.*, mucous gland; *o.*, ovary; *p.t.*, parenchymatous tissue; *s.d.*, samentasche (Sperm-pouch); *sem.rec.*, seminal receptacle; *sem.rec.o.*, oviduct; *t.c.*, tail cavity; *t.f.*, tail fin; *v.*, vagina; *ves.sem.*, seminal vesicle; *v.l.m.*, ventral longitudinal muscle; *v.p.n.*, ventral posterior nerve.

Sperm transference in *Spadella* is not reciprocal.¹ One individual in which the seminal vesicles are filled with spermatozoa approaches another in which the seminal receptacle is empty. The first individual then adjusts itself in such a position that its seminal vesicle is quite close to the vaginal opening of the other. The spermatozoa are then discharged in a mass and attached to the vaginal opening of the other. After this has been achieved, the first individual shifts its position in such a way that its head faces the tail of the second. In this new position they remain close together, lifting their heads simultaneously at intervals, so that the coronae ciliatae of both the individuals touch each other in this process. During this time the cilia in the vagina are very active and the sperms wriggle their way through the vagina into the seminal receptacle. One to two hours may be required for the entire mass of spermatozoa to pass into the seminal receptacle and the animals remain in the second position during the whole time, but if the seminal receptacle is filled up before the entire mass of spermatozoa has passed in, or if the individuals are disturbed, they swim apart and the second individual bends its head backwards and picks up and swallows the mass of sperm, which still remains on the external opening of the vagina.

Though the animals are hermaphrodites, during the time of sperm transference the one in which the seminal vesicles are full becomes distinctly active while the other remains passive.

12. DEVELOPMENT.

Material and Technique.—The development of *Spadella cephaloptera* was studied with captured specimens which were reared in the Laboratory of the Marine Biological Association during the summer months of 1930 and during

¹ The diagram of Vasiljev, 1925, showing the mode of sperm transference, does not represent the correct process. According to him ('*Biologia generalis*', p. 266) the two individuals come together in such a way that the opening of the seminal vesicle of the one touches the vaginal opening of the other and vice versa, suggesting that sperm transference is reciprocal. During the two years of my work on *Spadella* I have observed the process on many occasions but reciprocal transference has never been found to take place.

October 1931. The eggs after having been extruded are attached in clusters to the surface of the finger-bowls and proceed to undergo their development almost immediately. Egg laying usually takes place between 8 a.m. and 10 a.m., and blastulae are formed by about noon the same day, and the larvae are hatched out in about forty-eight hours. In order to get a complete series of the embryonic stages of development it was, therefore, necessary to collect the eggs at frequent intervals during day and night, but since the number of clusters obtainable each day was limited and uncertain, and since it was difficult to separate the eggs from the clusters, different stages were preserved on different days, lengthening the interval each day from the time of extrusion.

The material was mainly fixed in Bouin Dubosecq fluid, and the best sections were obtained from specimens which were fixed for about one hour and, after washing the fixative in 70 per cent. alcohol, treated with a few grains of lithium carbonate to remove traces of picric acid. Sections were made of all stages from early cleavage up to the 28th day after hatching. The early stages of development could be easily studied in the living embryos; after twenty-eight hours, however, the embryos become rather opaque and the structure more complicated, so that sectioning becomes absolutely essential. Double embedding with celloidin and paraffin was followed throughout with a slight modification of the usual method.

Specimens are passed from a mixture of absolute alcohol and ether through grades of celloidin, starting with a very thin solution. Apart from the fact that this method involves considerable time, there is also a great disadvantage, especially when the specimens happen to be embryonic stages which are covered by thin membrane and in which there is a wide central space such as the archenteric cavity. When these specimens are brought up to absolute alcohol and ether the central cavity gets filled up with the mixture of absolute alcohol and ether, and when these are transferred into a solution of celloidin, however thin it is, the difference in osmotic pressure between the solution of celloidin surrounding the specimens and the mixture of absolute alcohol and ether in the archenteric cavity

causes the embryos to shrink considerably and lose their natural shape. However long the specimens are left in this solution the original shape is not regained, probably because the covering membrane does not allow a free permeation of the celloidin. To overcome this, the mixture of absolute alcohol and ether was partially removed by means of a pipette, leaving about 1 c.c. of it in the tube with the specimens. To this was added sufficient quantity of a solution of 4 per cent. celloidin. As the ether and absolute alcohol does not mix with the celloidin immediately, it forms a top layer with the specimen resting on the surface of the layer of celloidin. As the ether and absolute alcohol slowly mixes with the celloidin, the slow gradations which result do not cause great difference in the osmotic pressure, and so the specimens do not shrink, but settle down to the bottom of the tube in about twenty-four hours. The celloidin in the tube is now removed and fresh 4 per cent. celloidin is poured in, and the specimens are then ready for the next process in about eight hours. Sections which were made through this process maintain the most perfect condition.

Development.—The egg of *Spadella* is about 0.3 mm. in diameter and is enclosed in a thin transparent membrane, which almost fits the egg. Between the egg of *Spadella* and *Sagitta* there are certain marked differences. We know that the eggs of *Sagitta* float on the surface of the sea and that they do not contain yolk granules to any noticeable degree; but in *Spadella* the eggs are attached to some foreign object below water level and contain minute yolk grains scattered uniformly in the cell substance. In spite of this difference it is found that cleavage is regular in both the forms. It is well established that the presence of large amount of yolk leads to an irregular and incomplete segmentation because the yolk is generally concentrated at one pole of the egg and interferes with the division of the protoplasm at this pole. As a result the animal pole segments rapidly, whilst the vegetative pole, where the yolk is concentrated, segments only very slowly. In the case of *Spadella*, however, the yolk is uniformly distributed through the egg, and so cleavage proceeds at the same rate in all parts. At Plymouth in its natural environment

Spadella is exposed to strong currents and extreme differences in the level of water owing to the ebb and flow of tide. Free-swimming animals are often swept down into the open sea, and the eggs are bound to be swept along also unless they are attached to some object. In this connexion it is interesting to note that eggs are usually laid when the tide is low. This naturally saves them from the strong currents of the ebb tide. The newly-hatched larva also resists the currents by adhering firmly to the smooth surfaces of sea-weeds by a pair of adhesive papillae on the head. The primitive free-swimming form probably possessed floating eggs, like its modern representative *Sagitta*, and therefore regular cleavage was the rule. The secondary adaptation to a coastal habitat, accompanied by the fixation of eggs, lengthened the period of development and consequently the eggs become yolky.

The first cleavages produce a spherical blastula with a well-marked but narrow blastocoele. The blastomeres are all of equal size and somewhat opaque owing to the presence of yolk. It has been stated that in *Sagitta* the blastocoele appears at the eight-celled stage, but in *Spadella*, at the eight-celled stage, the embryo is solid without any trace of blastocoele. Cleavage proceeds very rapidly, and during the next two divisions the first traces of a blastocoele are observed. Beyond this stage it is rather difficult to keep count of the number of cells, but it may be stated that during this time the blastocoele widens into a conspicuous spherical space surrounded by numerous cells of uniform size, thus resulting in a typical blastula. In about six hours after extrusion one side of the blastula flattens and invaginates into the blastocoelic cavity. As the invagination progresses the blastocoele slowly diminishes and the invaginated layer of cells comes into close contact with the inner surface of the outer layer of cells, thus obliterating the blastocoelic space completely. At the end of seven hours the embryo is a typical gastrula formed of two layers, the outer epiblast and the inner hypoblast, which encloses a wide space called the archenteric cavity with a narrow blastopore at one end. The pole which bears the blastopore marks the posterior end of the embryo. After this stage has been reached the further development is

comparatively slow, and before the next visible stage is reached a few changes take place which affect only the archenteron. The blastopore narrows down by degrees until its lips almost touch each other. The anterior end of the archenteric cavity widens considerably, and this is followed by a corresponding alteration in the shape of the embryo, which is now slightly elongated with a narrow posterior end. In the anterior end of the archenteric wall, slightly towards one side of the median line, one of the cells of the hypoblast begins to show certain marked changes, which can be easily observed in the living embryo. This cell enlarges and becomes more transparent than the surrounding cells. The archenteric cells surrounding it disconnect themselves from the sides of this large cell, and the dent on each side which separates it becomes more and more conspicuous. In about twenty minutes it disentangles itself from the surrounding cells and, moving to the inner surface of the layer of hypoblast, becomes a free cell in the archenteric cavity.

This cell, which is differentiated from the surrounding cells of the archenteron at this early stage in development, is the germ-cell. From the first appearance it is characterized by its large size and transparency. When first seen in the wall of the archenteron it is somewhat wedge-shaped, the broader end being directed inwards. Soon after the germ-cell becomes free in the archenteric cavity, it assumes a spherical shape and divides into two cells, which remain close together along the line of division. The archenteric cells close in and obliterate the dent which was left by the germ-cell, so that the wall of the archenteron becomes whole again without any sign to mark the original position of the germ-cell. Though the germ-cells are now free they still remain close to the place of origin at the anterior end of the archenteric cavity.

The first differentiation of the germ-cell is shown in fig. 22, Pl. 37, which is a longitudinal section of an embryo about eight hours old. At this stage the opening of the blastopore is very much reduced and the cells surrounding it have come into close contact. The side of the embryo opposite the locus of the blastopore represents the anterior end, and it will be seen that the archenteric cavity is very wide anteriorly and narrows down

towards the blastopore. The germ-cell is situated in the wall of the archenteron slightly towards one side of the median line at the anterior end. It is larger than the surrounding cells, and in Delafield's haematoxylin the protoplasm stains deeper and the cell is devoid of any trace of yolk. The spherical nucleus is at least four times as large as the surrounding nuclei, and the line of separation between it and the surrounding cells is clearly marked.

The transparent appearance of the germ-cell in the living condition is probably due to the absence of yolk granules. A glance at the figure shows that the ordinary cells of the epiblast and hypoblast are formed of two distinct parts, which take stains in different degrees. In the epiblast there is an outer region which stains deeply and contains the oval nucleus. The rest of the cell stains very lightly and is filled with minute granules. These two regions are the protoplasmic and the yolky part of the cell respectively. In the hypoblast the protoplasmic part forms the lining of the archenteric cavity, while the yolky part lies adjacent to the yolky part of the epiblast. In the germ-cell, however, this differentiation into two regions does not exist, so that the entire cell stains deeply.

In the next stage, represented in fig. 23, Pl. 37, the germ-cell (*gen.c.*) has moved inwards, and its basal end is in contact with the inner surface of the continuous layer of archenteric cells. This section was cut in a slightly obliquely longitudinal plane so that the blastopore is not seen. The germ-cell still has a wedge-shaped appearance, but the nucleus is already divided and the two daughter nuclei are situated wide apart. In the next stage (fig. 24, Pl. 37) the germ-cell has assumed an ovoid spherical form and has completely separated itself from the surface of the archenteric cells. There are two daughter nuclei, and the protoplasm surrounding them is divided by a faint median line which is much clearer in the living condition. The mother germ-cell is now divided into two distinct cells, and the daughter cells still remain attached to each other as a single mass until a later stage in development is reached¹.

While the germ-cells are developing, certain changes are also taking place in the archenteric wall, which ultimately give rise

to the coelom and the intestine. When the germ-cell is undifferentiated the archenteric cavity is continuous. It widens at its anterior end, and when the germ-cell shows the first signs of differentiation the archenteric wall at the anterior end, on each side of the median line, thickens slightly, marking the beginning of two inwardly directed folds. When the germ-cell has separated from the archenteric wall these thickenings are seen on each side of it. The commencement of the folds marks the beginning of the separation of the archenteric cavity into three parts. Between the two folds is an anteriorly directed median depression in which are situated the germ-cells. During the later stages this median anterior region of the archenteric cavity gives rise to the intestinal part of the archenteron, while behind the folds is seen a laterally directed arm-like projection of the archenteric cavity on each side. These lateral projections of the archenteric cavity, which are the result of the inward growth of the folds, are the rudiments of the coelomic pouches.

When the embryo is about fourteen hours old these three parts of archenteric cavity are clearly seen, and fig. 24, Pl. 37, shows them very distinctly. The blastopore has almost closed, though its position is marked by a string of deeply-staining cells. The germ-cell is in the middle part of the anterior region, and on each side of it are seen the rudiments of the archenteric folds (*arch.f.*). The laterally directed arm-like projections of the cavity are the coelomic pouches (*coel.p.*).

As the folds develop they grow backwards into the archenteric cavity as a double wall on each side of the median line. Each fold has two rows of nuclei. The outer row forms the inner lining of the coelomic pouch and later gives rise to the splanchnic epithelium, while the inner row forms the endodermal wall of the gut. As the folds continue to elongate, the three divisions of the archenteric cavity become more and more emphasized. At this stage the embryo elongates slightly so that the lumen of the coelomic pouches and the intestine becomes very narrow. The germ-cell, which was at first situated in the median anterior chamber, moves backwards, probably pushed along by the growing archenteric folds.

The embryo is now about sixteen hours old. The blastopore

is completely closed and no trace of its original position is visible either in sections or in the living condition. The archenteric folds have grown considerably backwards, and owing to the elongation of the embryo the cavities of the coelomic pouches and the intestine have become very narrow. The posterior end of the archenteric cavity is spacious and the germ-cells are now situated close to its wall (fig. 25, *gen.c.*, Pl. 37). The archenteric folds are still separate and do not touch the posterior boundary of the archenteric cavity, so that the coelomic pouches and the median chamber, i.e. the future lumen of the gut, are in open communication with the posterior part of the main archenteric cavity.

It is necessary to emphasize certain differences between the coelom formation of Chaetognatha and other invertebrates such as Echinodermata, as the two modes have been confused to a certain extent in attempting to explain the affinities of the former. In a typical case, such as *Asterias*, it is seen that the coelom originates as pouches on each side of the anterior end of the archenteric cavity by the outward growth of the wall of the archenteron. This soon becomes cut off as a closed vesicle on each side, while the greater part of the archenteron persists as the future gut. The fundamental difference between the coelom formation in the two groups is that while in Echinoderms the coelomic pouches are formed by the outward growth of the antero-lateral wall of the archenteron, in Chaetognatha the pouches originate by the development of posteriorly directed folds into the archenteric cavity. In Echinoderms, before the stomodaeum is formed, the connexion of the coelomic pouches with the archenteric cavity is at the anterior end and the pouches are directed backwards, but in Chaetognatha as the folds continue to grow backwards they carry backwards the openings of the coelomic pouches into the main archenteric cavity, so that when the folds are considerably developed as in the sixteen-hours' stage (fig. 24, Pl. 37) the openings of the pouches are really at the posterior end, and the pouches are directed forwards.

Some hours elapse before any further changes take place in the embryo. The two germ-cells now divide, resulting in four

daughter nuclei. One pair of daughter nuclei moves towards the right and the other towards the left side of the median line, and the two nuclei of each pair lie one behind the other close to the posterior end of the archenteric folds, in the main part of the archenteric cavity. When the embryo is about twenty-four hours old the posterior ends of the two folds come into contact, and the opening being thus occluded the intestine becomes a closed tube with a narrow lumen in the centre. The lateral coelomic pouches are still connected with the median posterior part of the archenteron. Fig. 25, Pl. 37, is a longitudinal section of an embryo at this stage. The posterior ends of the archenteric folds (*arch.f.*) have fused together and the intestine (*int.*) is seen as a closed median tube. The coelomic pouches (*coel.p.*) are very narrow and open posteriorly into the median part of the archenteron, in which the germ-cells (*gen.c.*) are situated.

The intestine now begins to elongate posteriorly, thus diminishing the area of the median part of the archenteric cavity, and the pair of germ-cells are pushed towards the right and left sides of the intestine. As growth proceeds the intestine touches the posterior wall of the archenteron and thus divides the single coelomic cavity into a right and left half, with one pair of germ-cells in each of the cavities. At the same time an ectodermal invagination develops in the anterior median line of the embryo and gives rise to the stomodaeum, which forms the future mouth and pharynx. The fusion of the posterior end of the intestine with the row of cells bounding the posterior border of the original archenteric cavity finally separates the right and left coelomic cavities. Fig. 26, Pl. 37, is a longitudinal section of an embryo at this stage. The stomodaeum (*st.*) has developed considerably and almost touches the anterior end of the intestine. The coelomic pouches, which were till now in open communication with the posterior part of the archenteron, are now separated into a right and left body-cavity (*b.c.*) by the meeting and fusion of the posterior end of the intestine with the posterior wall of the archenteron.

The gradual development and narrowing of the embryo causes the obliteration of the cavities. When the embryo is about

twenty-four hours old the lumen of the intestine gets filled up with non-nucleated protoplasmic material; a similar process also takes place in the coelomic cavities. Cell-limits are not visible in these protoplasmic masses, but outside the rings of nuclei cell outlines are very distinct. This condition leads to the same inference as that of Doncaster that the protoplasmic contents of the cavities are of a loose watery nature.

The stomodaeum is now connected with the anterior end of the intestine. At this stage a small constriction appears near the anterior ends of the coelomic sacs, and as the constriction deepens a small ring of mesoblast is separated from the anterior ends of the two body-cavities. These anterior masses of cells, which from their first appearance are devoid of any distinct cavity, later give rise to the mesodermal structures of the head. Fig. 27, Pl. 37, is a longitudinal section of an embryo about twenty-seven hours old. The stomodaeum (*st.*) is seen joining the median string of endodermal cells (*int.*), and the two rings of cephalic mesoderm cells (*b.c. 2*) are situated on each side of the posterior end of the stomodaeum.

Towards the anterior end of the main body-cavities (*b.c. 1*) the lateral ectoderm on the right and left sides of the embryo appears thick. The ectodermal nuclei in these regions multiply and sink inwards. It has already been stated that all the cells of the epiblast and hypoblast are clearly divided into a non-staining yolky part and a deeply-staining protoplasmic part. In the ectoderm up to this stage the protoplasmic part formed the outer surface, but when the nuclei begin to divide the protoplasmic area becomes thicker and extends inwards as converging strands (*g.c.*). This marks the origin of the ganglion cells. The ectodermal nuclei in these regions sink inwards through the protoplasmic strands and form the ganglion cells, which become more and more prominent during the later stages.

The embryo now shows all the different layers of the adult. The stomodaeum is connected with the intestine, the mesoderm is divided into cephalic and trunk cavities, and the ganglion cells are beginning to be formed. The embryo now begins to curve inside the egg-membrane, and the gradual elongation brings the different layers into such close contact that during

the further development observations of living embryos do not serve any practical purpose.

Soon after the beginning of curvature the intestinal cells become very thin and inconspicuous, and are seen only as a thin median septum, called the endodermal septum by Doncaster. The nuclei of the somatic mesoderm become aggregated into a dorsal and ventral mass on each side, which are connected with the lateral area only by thin strands of protoplasm from the outer side of the nuclei nearest to the lateral lines. These four masses of trunk mesoderm are seen in fig. 28, Pl. 37, which is a transverse section passing through the head and trunk of an embryo about twenty-eight hours old. The mesoderm appears as a solid circular area in the trunk, which is divided into a right and left half by the vertical endodermal septum, which is not quite clear in this particular section. The greater part of the trunk cavity is filled with the non-nucleated protoplasmic material, and the mesodermal nuclei are arranged in four rows, two on each side. Between the bases of the mesodermal cells and the nuclei the protoplasm is very clear and cell outlines are visible (*p.m.s.*), while the protoplasm on the inner side of the nuclei stains very deep, and it is this deeply-staining part which extends as thin strands towards the lateral line. The clear protoplasm forming the basal part of the mesodermal cells becomes modified into elongated strands and gives rise to the trunk-muscles after the embryo hatches out as the larva, while the nuclei and the outer deeply-staining protoplasm form the lining of the coelomic cavities.

The converging protoplasmic strands from the lateral ectoderm of the trunk are now very prominent and the ganglion cells are more numerous. In the head region the stomodaeum forms a median tube and the ring of cephalic mesoderm on each side is well developed. The lateral cephalic ectoderm is thick, and the nuclei, which are situated nearer to the mesodermal masses, are the ganglion cells which later give rise to the cerebral ganglia.

The embryo now elongates, the head becomes thicker and broader, and the tail tapers. The endodermal septum extends as a thin strand from the region of the neck to the posterior end

of the body, but when the tail region elongates still more the endodermal septum does not show a corresponding elongation, so that it is probable that the median septum of the tail is formed by the fusion of the inner lining of the right and left coelomic cavities along the median line. Near the anterior end of the tail, however, the extension of the endodermal septum between the two tail cavities is very clearly seen. As the embryo develops further this posterior extension into the tail degenerates, leaving no trace of it in the adult.

When the embryo is about thirty-five hours old the ganglion cells of the trunk are completely separated from the row of ectoderm cells and occur as two deeply-staining masses in the ventro-lateral region, close to the outer boundary of the mesoderm. The hood also begins to develop at this stage. According to Doncaster, in *Sagitta* the lateral cephalic ectoderm becomes two or three layers thick and constitutes the rudiment of the hood. Later on the dorsal surface the two outer rows split off from the inner row and form the hood. It has been shown by the author in a separate paper (John, 1931) that this explanation is not wholly in accordance with the adult anatomy. In *Spadella* the rudiment of the hood is first formed as a small outgrowth of the ectoderm from the thick lateral region. It grows ventralwards on each side overlapping the lateral wall of the head (fig. 29, *h.*, Pl. 38).

The further development of the embryo is not accompanied by any more differentiation of parts. It elongates slowly and curls through more than one full turn, so that in optical sections the tail overlaps the head. The ganglion cells multiply rapidly, and observation of living material at this stage shows them more prominent than the rest of the internal structure.

No further changes take place during the remaining period of embryonic development. The cells which are destined to form the different systems have all been differentiated, and the embryo now resembles the adult to some extent. The three regions of the body, head, trunk, and tail, could be distinguished. The ganglion cells, which develop into the visceral ganglion, the mesoderm which gives rise to the muscular system and the coelomic cavities, the germ-cells, and the thin band of endoderm

cells which develop into the alimentary canal, constitute the chief parts. The head becomes more conspicuous and the bands of ganglion cells increase in thickness. The edges of the hood meet and fuse in the ventral median line behind the mouth, and the sides of the tail become slightly flattened out on each side as the rudiments of the lateral fins. In this condition the embryo hatches out at the end of about forty-eight hours as a young larva.

The habits and structure of the newly-hatched young *Spadella* differ markedly from those of the adult, so that it is justifiable to use the term larva to describe it. A few hours before hatching the embryo shows signs of movement inside the egg-membrane. At the time of hatching the egg-membrane bursts, the larva wriggles out and straightens, and without any effort of its own sinks to the bottom of the finger-bowl and remains adhering by its anterior end. When all the eggs have hatched, either at the same time or successively, all the larvae which came out of the same cluster of eggs can be seen adhering to the empty egg-membranes on the same spot where the cluster of eggs was originally attached. They are not capable of rapid movement, but if disturbed with the fine end of a glass needle they detach themselves, wriggle a short distance, and come to rest again. During the first, second, and third day the larva shows very little which has not already been observed in the embryo. When hatched the larvae are about 1.5 mm. in length and are rather transparent, but as they do not move except when disturbed, and remain in groups, it is not difficult to detect them, especially with the aid of a pocket lens.

The essential difference between the larvae of *Sagitta* and *Spadella* at this stage is very characteristic of the mode of life of the adult. The young *Sagitta* is so transparent as to be almost invisible, and lies motionless near the surface of the water. When disturbed it swims in a jerky manner very like the adult, whereas the larvae of *Spadella* adheres to the surface on which the eggs were originally attached and is capable only of very little locomotion. It has already been pointed out that the attachment of the eggs and the capability of adhesion of the larvae are essential for survival in the kind of habitat in which *Spadella cephaloptera* is usually found.

Adhesion is effected by the development of adhesive cells on the ventral surface of the trunk (fig. 33, *ad.c.*, Pl. 38), and chiefly by the growth of a lateral adhesive process on each side of the anterior end of the head (fig. 32, *t.ad.*, Pl. 38). These adhesive papillae are cylindrical in shape and are composed of ordinary ectodermal cells, which at the tip are modified into a group of three or four strong adhesive cells.

The power of adhesion can be very easily observed by forcing a small jet of water against the larva, and if the current is directed from the posterior end, the tail will be lifted up by the current, but the oral end remains firmly attached so that the larva is not easily dislodged by the current.

The hood which was formed in the embryo as a lateral fold of the cephalic ectoderm on each side now grows rapidly and sheaths the postero-ventral surface of the head by the fusion of the right and left halves along the ventral median line, leaving an oval opening round the vestibule. The tail fin and lateral fins appear as a very transparent continuous fold of the ectoderm of the tail (fig. 20, *l.f.* and *t.f.*, Pl. 36) which extends to the posterior end of the visceral bands of ganglion cells (fig. 20, *v.g.c.*, Pl. 36).

During the first three days of the larval history certain changes also take place in the internal structure. The endodermal septum (fig. 20, *end.sept.*, Pl. 36) becomes thicker and more distinct than in the embryo, but the alimentary canal has not yet developed a lumen. The greater part of the cell-substance of the trunk and tail mesoderm is elongated into strands in the direction of the long axis of the body and transforms itself into four bands of longitudinal muscles. The oblique muscles are not seen at this stage in transverse sections, but in the lateral line between the dorso-lateral and ventro-lateral bands of longitudinal muscles a few cells are seen projecting into the coelom marking the region of insertion of the oblique muscles. The lateral ectoderm of the trunk is very thick owing to the presence of the large bands of ganglion cells. The ganglion cells form a prominent band of deeply-staining cells on each side, which extend from the neck to the posterior end of the alimentary canal (fig. 20, *v.g.c.*, Pl. 36). In the head an aggregation of ganglion cells can be seen at the anterior end, but these

are not yet differentiated into the brain and lateral ganglia. The cephalic mesoderm is still undifferentiated and occurs as a solid mass of cells on each side of the pharynx. In the postero-lateral region of the head, slightly towards the dorsal aspect, a small shallow depression arises below the hood on each side. This is lined with large ectodermal cells, from which the prehensile spines develop later.

The first sensory organ to make its appearance is the corona ciliata. On the fourth day after hatching, a shallow circular depression is visible in the dorsal ectoderm of the neck. The edge of this depression is lined by two rows of deeply-staining cells (fig. 20, *c.c.*, Pl. 36). When first formed the corona ciliata is perfectly circular in outline and cilia are not visible. The cells which give rise to the corona ciliata could be observed in the dorsal ectoderm of the neck from the first day after hatching. At first they are similar to the cells of the ectoderm, but, as development proceeds, become larger and deeply staining and form two concentric rings.

On the fifth day the alimentary canal becomes wider and a lumen develops inside it. In the mesoderm the transformation into muscle-bands is very marked, though the fibres are very few and rather indistinct in their arrangement. The muscle-bands are covered on their inner surface by a thin nucleated layer. This forms the lining of the coelom throughout life, and apart from this no separate peritoneal epithelium is developed.

The ganglion cells in the head have increased in number and now give rise to the rudiment of the brain, while at the sides of the mouth they group themselves into a rounded mass from which the vestibular ganglia develop later (fig. 20, *vest.g.*, Pl. 36). The eyes appear as two minute black spots on the dorsal surface of the head. They could be observed only in living specimens; when preserved they become indistinguishable and their structure could not be made out, even in sections. At this stage the ectoderm of the head and trunk shows small aggregations of deeply-staining cells, and these are the rudiments of the ciliated pits of the adult.

The characteristic network of parenchymatous tissue begins to develop at this stage. The origin of this tissue can be traced

from the early stages of development. It has already been stated that the ectoderm is comparatively thick and that each cell is formed of a deeply-staining outer protoplasmic area containing the nucleus and an inner lightly-staining part, which during the early stages contain minute yolk-granules (fig. 24, Pl. 37). The ectoderm remains as a single layer of cells until the embryo is about twenty-seven hours old. At this stage the protoplasmic part of the ectoderm cells in the lateral regions of the trunk becomes thicker and pushes into the non-staining part in the form of thin strands. The ectodermal nuclei proliferate, and some of them move inwards through the protoplasmic strands and ultimately become the ganglion cells (figs. 27 and 28, *g.c.*, Pl. 37). At about the thirty-five-hour stage the ganglion cells separate themselves from the ectodermal layer and aggregate into a mass of deeply-staining cells on each side of the ventral bands of mesoderm cells. Fig. 30, Pl. 38, is a highly magnified transverse section of the trunk. Two bands of mesoderm cells are seen on each side of the median endodermal septum, and the ganglion cells are situated close to the outer surface of the two ventral bands (fig. 30, Pl. 38, is drawn with the ventral side upwards so as to maintain the relation with the curvature of the embryo). The ectodermal area is relatively thicker on the ventral and lateral regions of the trunk. The part of the body outside the bands of mesoderm could be distinguished into three regions: the masses of deeply-staining ganglion cells; a wide lightly-staining area formed of loose watery protoplasm with a few scattered nuclei, situated on the outer side of the masses of ganglion cells; and the outer continuous ring of nuclei surrounded by a thin layer of protoplasm, which constitute the ectoderm proper. The loose protoplasmic area with a few scattered nuclei, situated between the masses of ganglion cells and the ectoderm proper, gives rise to the reticulated parenchymatous tissue. From its situation outside the masses of ganglion cells there can be no doubt that it is ectodermal in origin. During the earlier stages the mesoderm is clearly marked off from the ectoderm, and the ectoderm is formed of a single layer of cells, so that if the mesoderm buds off cells into this intermediate area it could be easily distinguished, but there is not

the faintest indication that such a thing takes place at any stage in development. The first signs of the proliferation of the ectoderm cells is when the ganglion cells develop. After the ganglion cells have sunk inwards and completely separated themselves from the ectodermal layer, some of the ectodermal nuclei on the ventral side divide again and the daughter nuclei sink into the intermediate area (fig. 30, *p.t.*, Pl. 38). During the early larval stages this intermediate area is completely separated from the ectoderm and forms a continuous layer surrounding the body. A few small nuclei are still visible, but they are in a state of degeneration (fig. 35, *p.t.*, Pl. 38). When the larva is about seven days old all the nuclei have disappeared and the protoplasm forms loose irregular strands. These irregular strands become more defined and the characteristic reticulate structure appears only after the larva has metamorphosed into the young *Spadella*.

From the fifth to the seventh day the larva does not increase in length to any appreciable extent, but during the seventh day considerable changes take place, giving rise to a type of structure closely resembling that of the adult animal. Up to this stage the visceral ganglion was represented by two longitudinal bands of ganglion cells with their edges placed vertically and having a semilunar shape in transverse sections (fig. 34, *v.g.c.*, Pl. 38). The ventral edges of the right and left bands are connected only by a mass of undifferentiated protoplasmic cell-substance lying beneath the median ventral ectoderm. This mass of cell-substance now begins to transform itself into the central fibrous mass of the ganglion called the central punktsubstanz (fig. 36, *v.g.punkt.*, Pl. 38), and this gives the visceral ganglion the appearance of a U in transverse sections. This completes the development of the visceral ganglion and gives it the structure found in the adult, with the only difference that while in the adult the visceral ganglion does not extend laterally beyond the ventral surface, in the larva the arms of the U embrace the sides of the muscular bands and extend to the base of the dorso-lateral body-wall. At the same time the central punktsubstanz also develops in the brain. The alimentary canal now becomes oval in transverse sections and a distinct wide

lumen appears inside it for the first time. As growth in the circumference of the alimentary canal is not followed by a corresponding growth in the width of the body, the spaces inside the coelomic cavities of the trunk are greatly reduced and the wall of the alimentary canal almost presses on the muscular bands (fig. 36, *int.*, Pl. 38). The anus is now formed at the posterior end of the alimentary canal, in front of the transverse septum in the ventral median line. From this it is evident that the larva begins to take food only from this stage onwards. The muscular bundles become thicker and the tendency towards the pinnate arrangement of the fibres becomes faintly visible. From the undifferentiated mesoderm of the head the cephalic muscles begin to develop, and from the lateral ectodermal depressions the prehensile spines grow in succession, but the number of spines could not be determined as they are invariably damaged while sectioning. The adhesive papillae on the head still persist, though all the adhesive cells on the ventral surface of the trunk have been absorbed. The neck changes considerably in shape and structure and assumes the condition exactly like that of the adult. Up to the sixth day it was circular in cross-section, the corona ciliata was in the form of two concentric circles on the dorsal surface, and the connective tissue was uniformly thick all round, but now the sides of the neck expand laterally into a short horizontal lobe on each side and the connective tissue at the sides becomes thicker. Following this expansion and flattening of the neck the corona ciliata also changes in shape and becomes oval, with the long diameter situated at right angles to the long axis of the body.

With the development of the cephalic muscles the head cavity becomes visible between the pharynx and the mesoderm on each side. The right and left cavities become continuous with each other above the dorsal surface of the pharynx by the coalescence of their adjacent walls, and thus a single unpaired cavity is formed.

The larva is now fully developed and is able to swim about and feed by itself on small diatoms and algae. It is still characterized by the presence of the antero-lateral adhesive papillae on the head and a few adhesive cells on the trunk. The length of the visceral ganglion in relation to the trunk indicates the

further growth of the larva. During the first seven days the ganglion extends from the neck to the posterior end of the intestine, but it later becomes restricted to the middle ventral region, being relatively shorter owing to differential growth. After the seventh day hardly any changes take place in the trunk or the tail except the further increase in length and the growth of adhesive cells on the anterior region of the ventral wall of the tail. This brings us to the fifteenth day after hatching. All through this period the most important character of the larva is its mode of adhesion by the anterior adhesive papillae and the adhesive cells on the ventral surface of the trunk.

Development of the Young Spadella.—Under artificial conditions most of the larvae died before they were fifteen days old, owing to the fact that they could not be properly fed. Every attempt to keep them alive failed, but on one occasion three specimens surmounted the mortality stage and developed into young Spadella. Of these three, one was mounted whole and the other two were sectioned. The following account of the young Spadella after metamorphosis is based on these three specimens. At the end of the fifteenth day, or thereabouts, the adhesive papillae on the head and the adhesive cells on the ventral wall of the trunk disappear, and this marks the transformation of the larva into a young Spadella, which attaches itself by the adhesive cells developed on the anterior end of the ventral wall of the tail and rests with the head slightly raised above the surface of attachment, exactly like the adult. It is now more vigorous and active and swims by darting movements. The full equipment of prehensile spines appears and the anterior teeth develop. The young Spadella now begins to feed upon small copepods.

It does not seem necessary to follow in detail the further growth of the young Spadella, except to mention a few important points. The alimentary canal still fills the greater part of the body-cavity (fig. 21, *int.*, Pl. 36), but the ventral ganglion is very much restricted and is now confined to the ventral surface of the trunk. The only other important structures which still remain to be described are the generative organs.

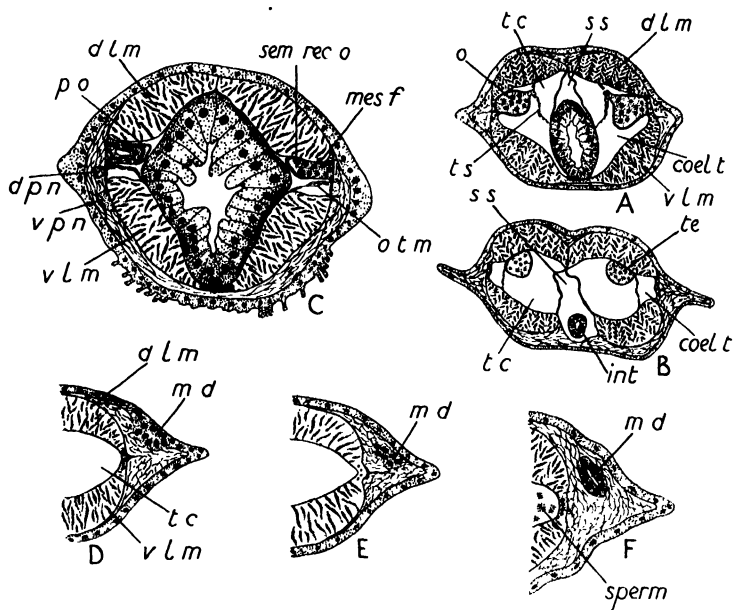
The early development of the germ-cells has been described

already (p. 672). The mother germ-cell gives rise to four cells which, still remaining close together, migrate towards the posterior end of the archenteric cavity. On reaching the posterior end they separate into two pairs, each of which passes into the corresponding coelomic pouch. The coelomic pouches are then cut off from the archenteric cavity by the meeting and fusion of the archenteric folds with the posterior wall of the archenteron. The right and left body-cavities are closed sacs, each of which contains a pair of germ-cells. These germ-cells are at first situated at the posterior end of the coelomic sac, but later they move forwards and occupy a position in the middle of the cavity, and the two nuclei divide into eight daughter nuclei. Fig. 37 A, Pl. 38, is a frontal section of an embryo about thirty hours old passing through the dorsal part of the curvature. (The orientation is shown in fig. 37 B, Pl. 38.) The four bands of cells indicate the future longitudinal muscles, and the germ-cells are embedded in the centre. As it is very difficult to get the correct orientation in the spherical embryos the eight daughter nuclei appear only on one side of the figure. The eight nuclei occur in two groups of four each, and are so arranged that one group lies behind the other, each group of four is embedded in a common mass of protoplasm. The genital rudiment has now the appearance of an ovoid body lying close to the intestine with its long axis parallel to the long axis of the body. It does not undergo any further changes till the larva is hatched. Up to the end of the third or fourth day the trunk coelom is filled with the loose protoplasmic substance already described, and the genital rudiments are embedded in it, but on the fourth day or thereabouts (time depending on the temperature under which the larva is reared) this solid condition of the mesoderm disappears and a continuous body-cavity, bounded by a somatic and a thin splanchnic layer, is established. These trunk-cavities surround the intestine and extend from the posterior end of the head to the tip of the tail. The genital rudiment, which is enclosed by a thin membrane, now moves across the body-cavity and comes to lie close to the body-wall. Along the line of contact the membrane enclosing the genital rudiment attaches itself with the body-wall. Each genital

rudiment lies rather obliquely so that its anterior end is attached to the lateral line (Text-fig. 5 A, *o.*), while the posterior end is situated more dorsally in contact with the inner surface of the dorsal longitudinal muscles (Text-fig. 5 B, *te.*). The further development of the genital organs is correlated with the formation of the secondary septum which divides the body-cavity into a distinct trunk and tail cavities. The origin of this septum is by no means clear, owing to the fact that it has been difficult to rear the larva to the metamorphosing stage. Doncaster (1902) has suggested that it might arise as a fold of the splanchnic mesoderm, or by the coalescence of the cellular envelopes in which the germ-cells are enclosed. The latter explanation assumes that the germ-cells are two distinct masses placed one behind the other and each enclosed by a membrane, and that they are attached during their early stages to the side of the intestine, so that as they move across the body-cavity their envelopes are elongated transversely. This view does not seem probable, at least so far as the development of *Spadella* is concerned, because in *Spadella* the genital rudiment divides into two parts only after it has attached itself to the body-wall, and secondly, it is never attached to the wall of the intestine, though during the early stages it lies close to the latter. Therefore the only other possibility is that the transverse septum develops as a fold of the coelomic epithelium.

The septum is first visible in the larva when it is about fifteen days old (fig. 20, *t.s.*, Pl. 36). It runs in an oblique direction from the posterior end of the intestine to the body-wall. When the septum is formed the genital rudiment is divided into two parts, an anterior part which is attached to the lateral line in front of the septum, and a posterior part (Text-fig. 5 B, *te.*) attached to the inner surface of the dorsal longitudinal muscles. The anterior part gives rise to the ovary and the posterior part develops into the testis. The completion of the septum between the trunk and tail marks the change from the larval to the adult structure. It still differs from the latter in many important respects—for example, the ovaries and the testes are each represented by a single cell and there are no genital ducts, the visceral ganglion is very large, the corona ciliata is circular,

and the tail fin is continuous with the lateral fin. The changes, however, which transform the young of this stage into the adult condition is very gradual, extending over several days. Figs. 20



TEXT-FIG. 5.

The development of the male and female ducts in the young *Spadella*. Figs. A and B. Showing the formation of the transverse septum and the attachment of the ovaries and testis. C. Section through a young *Spadella* about 24 days old showing the origin of the seminal receptacle. D, E, and F. Transverse section through the posterior region of the tail, showing the different stages in the development of the male duct. *coel.t.*, trunk coelom; *d.l.m.*, dorsal longitudinal muscle; *d.p.n.*, dorsal posterior nerve; *int.*, intestine; *mes.f.*, mesodermal fold forming the seminal receptacle; *m.d.*, male duct; *o.*, ovary; *o.t.m.*, oblique transverse muscle; *p.o.*, posterior end of the ovary; *sem.rec.o.*, opening of the seminal receptacle; *s.s.*, primary body-cavity between the median septa; *t.c.*, tail cavity; *te.*, testis; *t.s.*, transverse septum; *v.l.m.*, ventral longitudinal muscle; *v.p.n.*, ventral posterior nerve.

and 21, Pl. 36, give a comparative idea of a well-advanced larva and a young *Spadella*. In the latter the visceral ganglion is very much reduced, ciliated pits are well developed the lateral fins are separated off from the tail fin by a narrow constriction,

in which the seminal vesicles are being formed, and the genital rudiments are already divided to form groups of smaller cells. In each group all the cells seem alike with prominent nuclei, and at this stage the male and female ducts begin to develop.

The origin and development of the genital ducts in Chaetognatha were first described by Grassi (1889) and later confirmed by Doncaster (1902). When the testis has increased considerably in size it begins to give off masses of sperm mother-cells into the tail-cavity, where they undergo their further development. At about the same time the male genital duct begins to arise in the narrow constriction, which forms the junction between the tail and lateral fin (fig. 21, *res.sem.*, Pl. 36). It first appears as a thickening of the dorso-lateral ectoderm (Text-fig. 5 D, *m.d.*). The cells which form the thickening sink into the connective tissue and form a solid rod of cells (Text-fig. 5 E, *m.d.*). At a later stage the cells separate to form the lumen (Text-fig. 5 F, *m.d.*). This tube now lies between the ectoderm and the mesoderm and traced forward it is found to taper into a narrow canal. At first there is no connexion with the mesoderm, but ultimately the narrow anterior end grows forward and joins the lateral coelomic epithelium so that the canal opens internally into the tail-cavity. It is at this point that the coelomic epithelium becomes modified into the genital funnel (coelomostome). As the animal reaches maturity the posterior end of the canal widens and forms the seminal vesicle. There can be no doubt that the seminal vesicle is ectodermal in origin and the short narrow canal, from its continuity with the seminal vesicle, seems to be of the same origin, but the genital funnel is undoubtedly derived from the coelomic epithelium.

The female germ rudiment lies just in front of the transverse septum close to the lateral line. After the stage is reached in which the rudiment of the ovary is a mass of similar cells, it grows forward and becomes a cylindrical organ covered by a thin layer of epithelium. At the same time a fold of mesoderm develops from the region of the lateral line in the posterior end of the ovary (Text-fig. 5 C, *mes.f.*). The two layers of the fold separate, giving rise to a narrow lumen, which develops into the seminal receptacle, and its anterior end narrows down and joins the

hind end of the ovary, so that the ovary opens directly into it (Text-fig. 5 c, *sem.rec.o.*). As growth proceeds the seminal receptacle enlarges, and from its anterior end a narrow tube develops below the opening into the ovary and extends forwards along the ventral side of the ovary. This anterior extension of the mesodermal fold, in which a narrow lumen becomes visible at a later stage, can be compared to the 'samentasche'. The origin of the seminal receptacle and 'samentasche' is identical with that of *Sagitta* (Stevens, 1910), though there is considerable difference in the extension of the eggs in the two types (cf. adult anatomy). As the animal matures the ectoderm in the posterior region of the seminal receptacle becomes thick and forms a narrow invagination which grows inwards and joins the seminal receptacle as the vagina, while the cells surrounding the external opening of the vagina become elongated and develop into the cement gland.

13. SUMMARY OF NEW POINTS.

1. The habits, structure, and development are described fully. No previous description of either development or habits has been given, and there are only a few scattered references to points of structure.

2. *Spadella cephaloptera* can withstand reduced salinity as experimentally proved, showing its adaptation to life in bays and sounds in the mouths of rivers.

3. The structure of the ciliated pits scattered over the body, of the mucous glands, cement glands, and adhesive cells has been described. It has also been shown that the ciliated pits are arranged in a regular order on the body.

4. The structure and function of the cement glands are described for the first time.

5. It has been experimentally proved that the corona ciliata is a tactile organ.

6. The position of the vestibular ganglion and its nerves is different from that in *Sagitta*.

7. The posterior nerves divide into two branches, the posterior dorsal and the posterior ventral, which are situated one on each side of the base of the amorphous basal substance of the fins.

8. When the mouth is opened the vestibule is evaginated and the mouth assumes a terminal position and projects forward.

9. The lateral plates play an important part in opening the mouth and support its sides. They function as true jaws.

10. The working of the muscles for opening the mouth and evaginating the vestibule has been fully described.

11. There are two series of oblique muscle-strands in the trunk-cavity.

12. Behind the posterior end of the alimentary canal there is a median space in the septum dividing right and left coelomic cavities which was supposed to be part of the trunk-coelom, but it has been shown that this is a primary body-cavity.

13. The wall of the alimentary canal is thick and not vacuolated and contains ridges due to the presence of large cylindrical cells.

14. The ovary opens directly into the seminal receptacle.

15. There is a distinct tube called the vagina which is ciliated. This opens into the seminal receptacle and its external opening is surrounded by the cement gland.

16. During sperm transference one animal in which the seminal vesicle is ripe approaches another and attaches the mass of sperms to the external opening of the vagina. It then turns round with its head facing the tail of the second individual and remaining close to each other both lift their heads at intervals. The corona ciliata of both the animals touch in this process. They remain in this position till the entire mass of sperms has passed into the seminal receptacle of the second animal. Sperm transference is not reciprocal.

17. Though the egg contains yolk, cleavage is regular owing to the fact that the yolk is uniformly distributed.

18. The general account of the sequence of early embryonic stages, previously based on a study of whole mounts, has been verified with sections.

19. The germ-cell is formed at the anterior end of the archenteron, before the archenteric folds appear.

20. The two germ-cells in each of the coelomic pouches are surrounded by a common envelope and are separated into the distinct ovary and testis only by the formation of the secondary septum.

21. The secondary septum is an oblique septum which originates from the mesoderm between the dorsal and ventral muscular bands of the somatic wall of the coelom.

22. The hood is formed as a lateral fold on each side of the mouth and not by the splitting of the lateral ectoderm as supposed by Doncaster.

23. The larva when hatched attaches itself to foreign objects by a pair of adhesive papillae on the sides of the anterior end of the head, which disappear later. (These are not the same as the tentacles of the adult.)

24. Adhesive cells on the ventral surface of the tail develop only when the larva is ten days old.

25. The parenchymatous tissue on the ventral side of the body develops from the ectodermal layer—the ectodermal cells proliferate and the daughter cells pass inwards. This tissue intervenes between the ventral ectoderm and the posterior end of the ganglion.

26. The male duct is partly formed from the ectoderm and partly from the coelomic epithelium.

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EXPLANATION OF PLATES, 34-38.

LIST OF ABBREVIATIONS.

ad.c., adhesive cells; *amor.b.s.*, amorphous basal substance; *an.*, anus; *arch.*, archenteron; *arch.f.*, folds of the archenteron; *at.*, anterior teeth; *b.*, brain; *b.c.*, body-cavity; *b.c.1.*, head coelom; *b.c.2.*, trunk coelom;

b.g., ganglion cells of the brain; *bl.*, opening of the blastopore; *b.m.*, base-membrane; *b.pnkt.*, punksubstanz of the brain; *c.c.*, corona ciliata; *c.g.*, cement gland; *c.m.*, cephalic muscle; *coel.h.*, head coelom; *coel.t.*, trunk coelom; *co.n.*, coronal nerve; *coel.p.*, coelomic pouches; *c.p.*, ciliated pit; *d.l.m.*, dorsal longitudinal muscles; *d.n.*, dorsal nerve; *d.p.n.*, dorsal posterior nerve; *ect.*, ectoderm; *end.sept.*, endodermal septum; *f.c.*, frontal commissure; *g.c.*, ganglion cells; *gen.c.*, genital cells; *h.*, hood; *in.op.v.*, internal opening of vagina; *int.*, intestine; *int.div.*, intestinal diverticulum; *lab.n.*, labial nerve; *l.f.*, lateral fin; *l.l.*, lateral line; *l.m.*, longitudinal muscular strands of the trunk; *l.n.*, lateral nerve; *l.o.e.n.*, lateral oesophageal nerve; *l.p.*, lateral plate; *l.p.p.*, lateral part of the lateral plate; *m.*, mouth; *m.ad.*, muscle adductor uncinorum; *m.b.*, muscle bicornis; *m.c.*, main commissure; *m.c.l.*, muscle complex lateralis; *m.c.o.p.*, muscle constrictor oris primus; *md.n.*, mandibular nerve; *m.d.v.e.*, muscle dilator vestibuli externus; *m.d.v.i.*, muscle dilator vestibuli internus; *m.e.s.*, muscle expansus superior; *mes.*, mesoblast; *mes.c.*, cephalic mesoderm; *mes.f.*, mesodermal fold forming the seminal receptacle; *m.g.*, mucous gland; *m.o.e.c.*, oesophageal circular muscle; *m.o.l.*, muscle obliquus longus; *m.o.s.*, muscle obliquus superficialis; *m.r.p.r.*, muscle retractor preputii; *m.s.*, median longitudinal septa; *m.t.d.*, muscle transverse dorsalis; *m.t.v.*, muscle transverse ventralis; *n.r.*, nerve to the muscle retractor preputii; *o.*, ovary; *oe.c.*, oesophageal commissure; *oe.g.*, oesophageal ganglion; *oes.*, oesophagus; *o.n.*, optic nerve; *o.t.m.*, oblique transverse muscle of the trunk; *p.m.s.*, protoplasmic strands forming the muscles; *p.n.*, posterior nerve; *p.s.*, prehensile spines; *p.t.*, connective tissue; *s.d.*, sementasche (sperm pouch); *sem.rec.*, seminal receptacle; *sem.rec.o.*, oviduct; *sp.*, ripe spermatozoa; *sperm.*, sperm mother-cells; *s.s.*, primary body-cavity between the median septa; *st.*, stomodaeum; *t.*, tentacle; *t.ad.*, adhesive tentacle; *t.c.*, tail cavity; *te.*, testis; *t.f.*, tail fin; *t.mes.*, trunk mesoderm; *t.s.*, transverse septum; *v.*, vagina; *ves.sem.*, seminal vesicle; *vest.*, vestibule; *vest.g.*, vestibular ganglion; *v.g.*, ventral ganglion; *v.g.c.*, visceral band of ganglion cells; *v.g.pnkt.*, punksubstanz of the visceral ganglion; *v.l.m.*, ventral longitudinal muscle; *v.n.*, vestibular nerve; *v.o.e.n.*, ventral oesophageal nerve; *v.p.n.*, ventral posterior nerve; *v.p.v.*, ventral posterior border of the vestibule.

PLATE 34.

Figs. 1-8.—Series of representative transverse sections through the head and collar regions.

PLATE 35.

Figs. 9-16.—Continuation of the series of transverse sections through trunk and tail.

PLATE 36.

Fig. 17.—Ventral view of the head showing the position of the mouth when opened and its relation to the lateral plates.

Fig. 18.—Dorsal view of the head showing the position of the lateral plates, hood, anterior teeth, and prehensile spines, when the mouth is opened.

Fig. 19.—Ventral view of the head when the mouth is closed.

Fig. 20.—Larva about fifteen days old.

Fig. 21.—Young *Spadella* about thirty days old.

PLATE 37.

Fig. 22.—Longitudinal section of gastrula showing the origin of the genital cell.

Fig. 23.—Section of a gastrula (obliquely longitudinal) showing the genital cell in the archenteric cavity.

Fig. 24.—Longitudinal section of a late gastrula (eighteen hours old) showing the origin of the archenteric folds.

Fig. 25.—Longitudinal section of an embryo about twenty hours old showing the archenteric folds, the origin of the intestine, the position of the genital cell, and the coelomic pouches.

Fig. 26.—Longitudinal section of an embryo about twenty-four hours old showing the separation of the right and left body-cavities and the origin of the stomodaeum.

Fig. 27.—Longitudinal section of an embryo about twenty-seven hours old showing the separation of the head coelom.

Fig. 28.—Transverse section of an embryo passing through the head and trunk showing the origin of the ganglion cells and the mesoderm cells arranged in four rows.

PLATE 38.

Fig. 29.—Transverse section of an embryo thirty-five hours old showing the development of the hood.

Fig. 30.—Transverse section of an embryo about thirty-five hours old, through the trunk, showing the position of the genital cells.

Fig. 31.—Transverse section of an embryo about thirty-eight hours old showing the structure of the trunk.

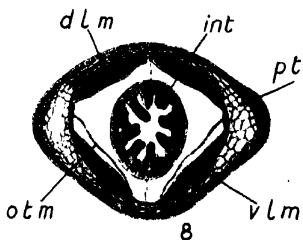
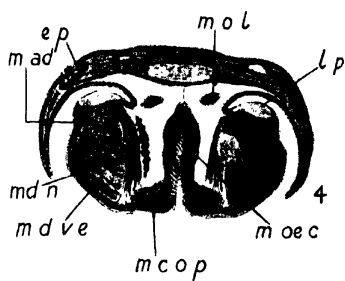
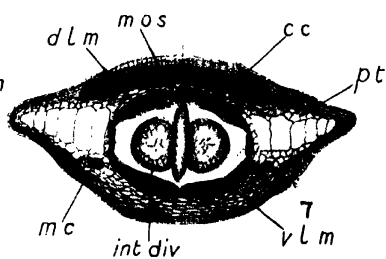
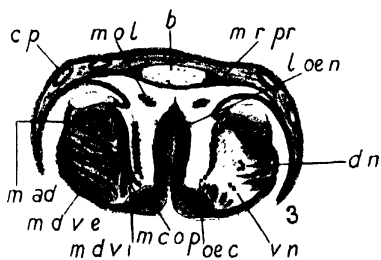
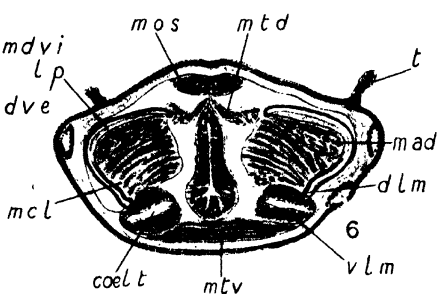
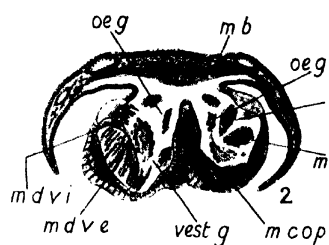
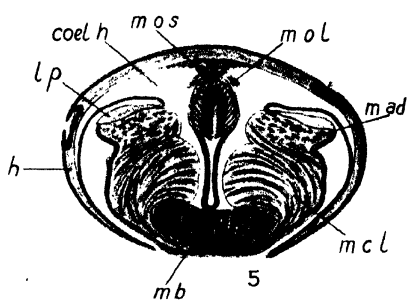
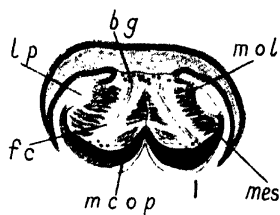
Fig. 32.—Transverse section through the anterior end of the head of a larva about five days old, showing the position of the adhesive papillae.

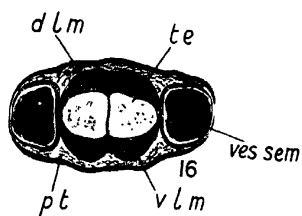
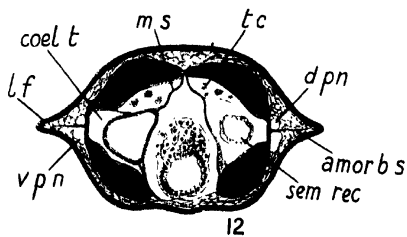
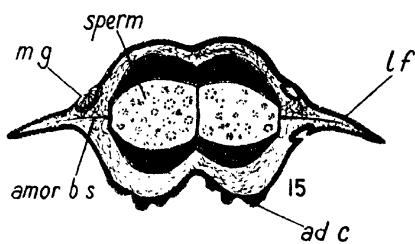
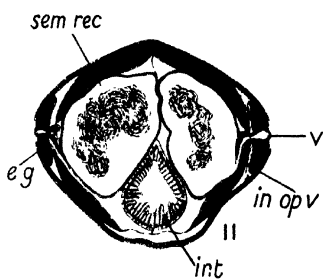
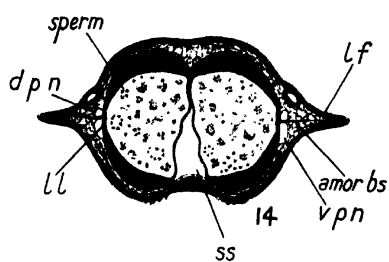
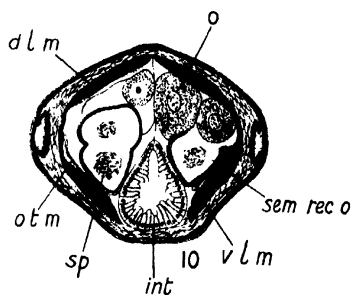
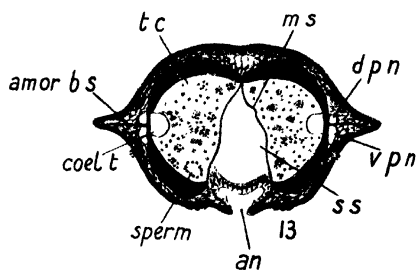
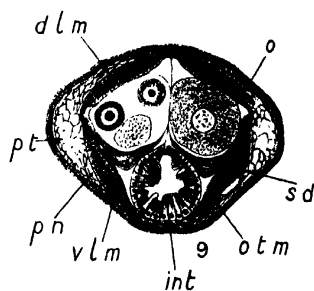
Fig. 33.—Section of the adhesive papillae on the head showing the adhesive cells at the tip.

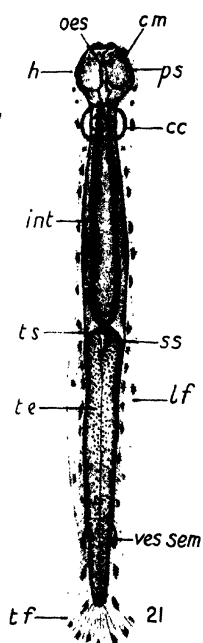
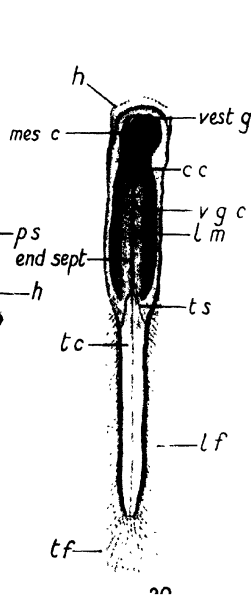
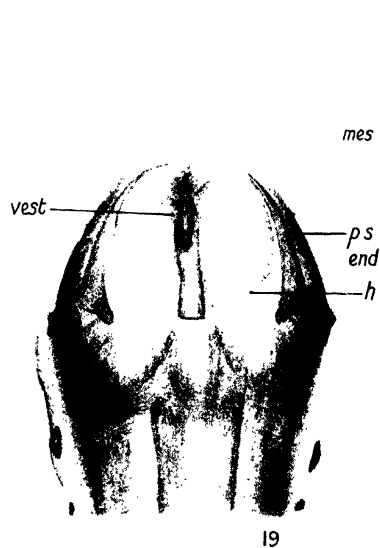
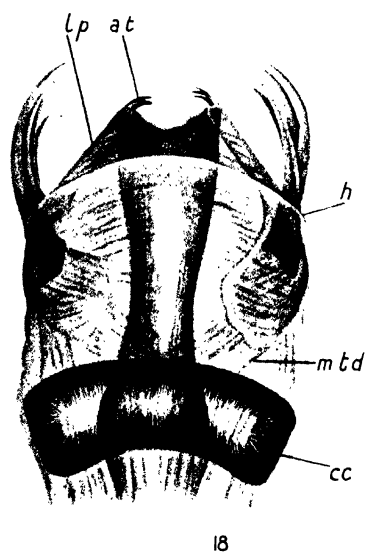
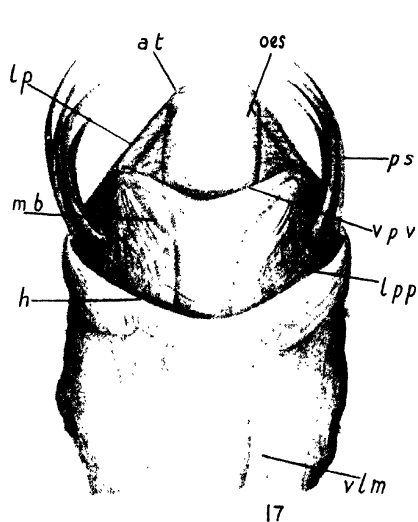
Figs. 34 and 35.—Transverse section of the larva, about seven days old.

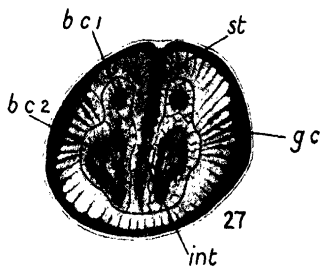
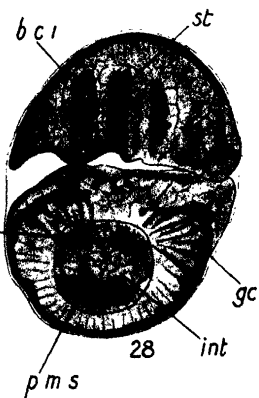
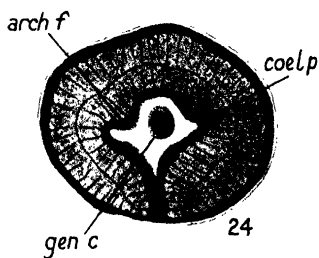
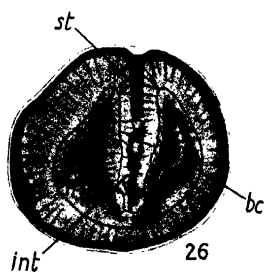
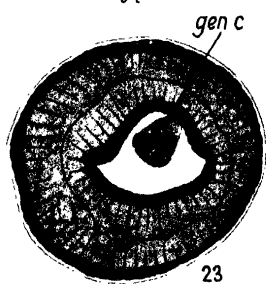
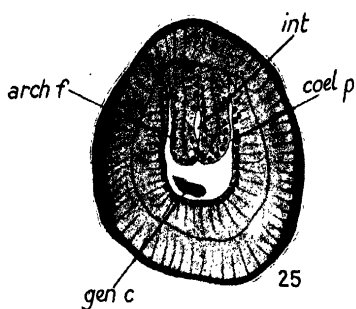
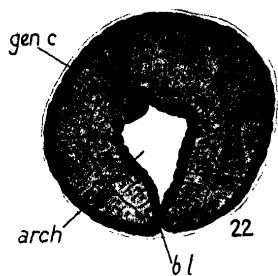
Fig. 36.—Transverse section of the larva, about twelve days old, passing through the visceral ganglion.

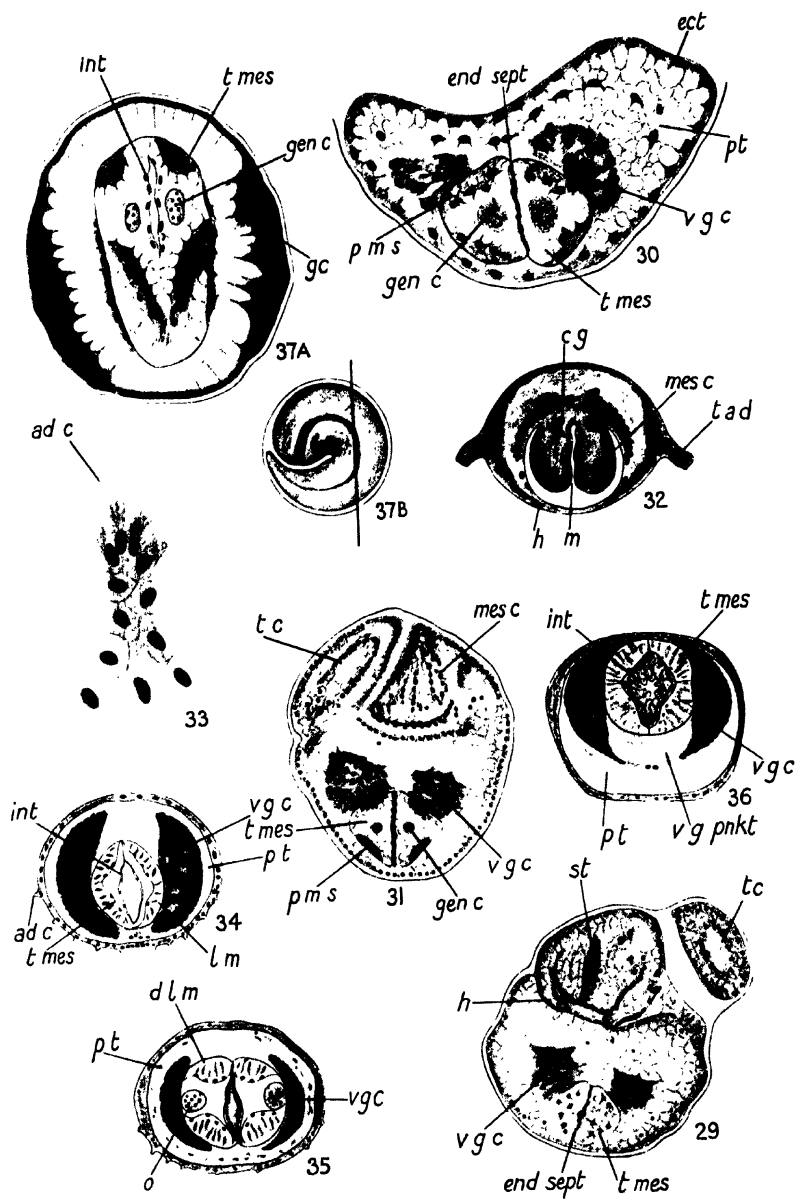
Fig. 37.—Frontal section of the embryo, passing through the dorsal part of curvature, showing the position of the germ-cells. Fig. 37 b shows the orientation of the same.











A Study of the Cytoplasmic Inclusions and Nucleolar Phenomena during the Oogenesis of the Mouse.

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With Plates 39-40 and 9 Text-figures.

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I. INTRODUCTION AND PREVIOUS WORK.

THE present investigations were undertaken in order to determine the history of the cytoplasmic inclusions and the behaviour of the nucleolus during the growth of the oocyte of the mouse (*Mus musculus*). As the mouse ovum contains but little yolk it was thought that an investigation of the ovarian oocyte and the early stages of cleavage, using silver and osmic techniques, might throw further light on the behaviour of the Golgi bodies and mitochondria of the vertebrate egg.

Kingery (18) records that in the mouse ovary there is an embryonic proliferation of cells from the germinal epithelium and a second proliferation commencing in the young animal of about one to two days old and continuing almost to sexual maturity. The cells from the first proliferation degenerate and are resorbed, while those of the second form the definitive ova.

The development of the oocyte is divided into three stages: Stage A includes the very early oocytes derived from the germinal epithelium. Stage B is distinguished by a slight increase in the size of both cell and nucleus. Stage C is marked by further increase in the size of the oocyte: a follicle wall is usually present. The cell remains in this stage until ready for maturation.

According to Kingery (op. cit.) ovaries fixed in Helly's, Zenker's, and Benda's fluid and mordanted in potassium bichromate, or fixed in Benda's fluid and subsequently stained in copper-haematoxylin, revealed the mitochondria as granules situated round the nuclei of the germinal epithelial cells. As the oocytes increase in size the mitochondria come to lie in one end of the cell, forming a crescent-shaped mass; at the beginning of Stage C they are distributed, as granules, uniformly throughout the ooplasm. The mitochondria of the follicle-cells are described as threads, rods, and granules. Kingery does not deal with yolk-formation.

Lams and Doorme (21) do not deal with the early stages of oogenesis of the mouse; consequently the youngest ovum to be described and figured is of considerable size and is surrounded by follicle-cells. The mitochondria are revealed as (osmic fixation followed by iron-haematoxylin) large granules collected in groups or scattered singly through the ooplasm, and also as fine granules many of which form short chains. In a later ovum, which is undergoing maturation in the oviduct, the majority of the mitochondria are figured as collected into fairly compact masses distributed through the ooplasm; the individual granules, however, do not appear to be as large as in the earlier oocytes.

Globules occur at the periphery of the nearly fully formed oocytes; these are faintly brown-black after osmic acid, but appear as clear spaces after corrosive-acetic fixation. They are identified as fat-globules. In ova, undergoing maturation in the oviduct, plastic and deutoplasmic zones are present; the fat-globules and the majority of the mitochondria are situated in the latter. After the entrance of the sperm the mitochondria, although still in preponderance in the deutoplasmic zone, tend to be more evenly distributed in the cytoplasm.

In an egg containing a sperm nucleus a body coloured red

by safranin and deep blue by haematoxylin is figured. This body is identified as a 'pseudochromosome' such as is described by Van der Stricht (27) as giving rise to mitochondria in the egg of the bat. In a slightly more advanced mouse ovum numerous small bodies, similar in staining reactions to the large 'pseudochromosome' of the earlier egg, are present. Lams and Doorme consider that the 'pseudochromosomes' result from a condensation of mitochondria, and that at a later stage they break up to form mitochondria again.

Branca (5), referring to the ooplasm of the mouse egg, mentions that his observations agree with those of Lams and Doorme. His figures of oocytes in atretic follicles show granules and bodies which, apparently, correspond to the mitochondria and fat-globules respectively of Lams and Doorme.

Kingery (19), dealing with follicular atresia in the ovary of the mouse, states that the cytoplasm of degenerating eggs stains more deeply than in the normal, and that numerous fat-globules are present. He does not describe Golgi bodies or mitochondria.

From the above account it will be seen that although certain aspects of mouse oogenesis have been worked out in detail no work has been carried out on the Golgi apparatus or on the details of yolk-formation. Thus Lams and Doorme give a detailed account of oogenesis in the mouse, but owing to the fact that their researches were carried out at a time when work on the Golgi apparatus had not been greatly developed, there remains room for further inquiry into the form and behaviour of the cytoplasmic inclusions. Kingery's papers, although of more recent date, are concerned chiefly with the growth of the oocyte, the nucleus and related phenomena, and with atresia. Consequently, he does not appear to have employed technique suitable for the demonstration of the Golgi apparatus.

II. MATERIAL AND METHODS.

For the study of the Golgi apparatus and mitochondria, ovaries were fixed according to the methods of Cajal, D'Amo, Mann-Kopsch, Kolatchev, and Flemming (without acetic).

The investigation of the nucleolar phenomena and of yolk-formation was carried out in material fixed in Flemming, Carnoy, and corrosive-acetic fixatives. Certain ovaries were treated according to Ciaccio's method for the identification of fats. As a further test for the presence of fats an ovary, fixed in Bouin's picro-formol, was treated with ether and subsequently stained in iron-haematoxylin and counter-stained with eosin.

Certain mice were killed at short intervals after pairing, and the upper part of the oviducts fixed in Cajal, Mann-Kopsch, Bouin, and Carnoy fixatives.

In all cases the material was dissected out as speedily as possible and immediately placed in the fixing fluid. For the study of the Golgi apparatus and mitochondria, sections were cut 3μ and 5μ in thickness; the other material was cut in sections 5μ and 8μ in thickness.

The greater part of the present work was carried out in the Department of Natural History, University College, Dundee, while the investigations on the tubal eggs and a certain part of the work on the Golgi apparatus and nucleolar phenomena was worked out in the Department of Zoology, University of Edinburgh. I wish, therefore, to express my thanks for research facilities granted in these Departments. That part of the work carried out at Edinburgh University was aided by a grant from the Earl of Moray Endowment of the University of Edinburgh.

III. OBSERVATIONS.

1. The Golgi Apparatus and Mitochondria.

(a) Oocytes.

For the demonstration of the Golgi apparatus and mitochondria Cajal's uranium nitrate and silver nitrate technique was found to be most satisfactory. In material treated by this method both Golgi bodies and mitochondria could be identified with ease. Consequently, the following account, unless where otherwise stated, refers to tissue treated according to Cajal's technique.

In the early oocytes, which are situated in and below the germinal epithelium and have not yet acquired a definite follicle wall, a dark mass of material occurs at one pole of the nucleus, while smaller masses of similar appearance are scattered through the ooplasm (fig. 3, Pl. 39). Dark masses occupy similar positions in preparations treated by the Da Fano, Mann-Kopsch, and Kolatchev methods. Owing to the structure and disposition of these bodies, and to their reaction to silver and osmic techniques, they are identified as the Golgi apparatus.

The juxta-nuclear Golgi apparatus of the young oocytes consists of rods and granules closely massed together, so that in many cases part of the apparatus appears as a solid body. This solid appearance, however, is, in all probability, a fixation effect. Most of the smaller masses of Golgi material scattered through the ooplasm are closely similar to the larger, while a few seem to consist of single rods (fig. 3, Pl. 39).

The mitochondria are revealed, in untuned sections, as small granules of a golden-brown colour distributed in the ooplasm but especially numerous round the large mass of Golgi material (fig. 3, Pl. 39).

In the older oocytes, which have acquired a follicle wall consisting of a single layer of cells, the Golgi material is clumped at one pole of the nucleus so as to form a juxta-nuclear body similar in structure to that of the earlier oocytes (fig. 5, Pl. 39). Only a few rods and granules remain outside this mass. On comparing this condition with that of the younger cells described above, it is seen that the majority of the smaller masses of Golgi material have joined the larger, thus forming a dense juxta-nuclear body.

At this stage the mitochondria are scattered through the ooplasm but appear to be more numerous in the vicinity of the nucleus and Golgi apparatus.

In slightly older oocytes the Golgi apparatus begins to break up into the individual rods and granules of which it is composed. These become distributed through the ooplasm (figs. 6 and 7, Pl. 39), although in certain cases they remain more numerous, until a later stage of oogenesis, in that part of the ooplasm originally occupied by the juxta-nuclear body.

The mitochondria are now fairly evenly distributed in the ooplasm except at the periphery, where they are less numerous.

In the next stage the Golgi bodies are distributed through the ooplasm in the form of large granules or thick rods, while most of the mitochondria are collected into clumps (fig. 8, Pl. 39). In late oocytes, with a follicle wall of several cell-layers, the mitochondria are gathered into clumps, leaving the intervening ooplasm practically free of these bodies. The Golgi bodies occur distributed through the cell and on and around the mitochondrial clumps (fig. 9, Pl. 39). In the later oocytes in mature follicles this tendency of the Golgi material to collect round the groups of mitochondria is more marked; in many cases the mitochondria are difficult to observe owing to the number of the Golgi bodies around the clumps. In these oocytes the Golgi bodies appear to be more numerous than in the earlier cells (fig. 9, Pl. 39).

Additional evidence in favour of the above findings was produced by an examination of the material treated by the Da Fano and osmic techniques. The Da Fano preparations revealed oocytes at different growth stages, in which the form and disposition of the Golgi bodies and mitochondria agreed closely with the above account. In Mann-Kopsch and Kolatchev material the mitochondria were not so well shown as by the silver methods. They demonstrated, however, that while the mitochondria of the early oocytes are scattered through the ooplasm, those of the older cells occur in clumps. The Golgi material was revealed as deeply osmophil masses in close association with the nucleus of the very young cell, but in the later oocytes distributed as granules or rods occupying similar positions to those of the silver preparations.

In ovaries fixed in Flemming's fixative (Gatenby's modification) and subsequently stained in iron-haematoxylin and light green, the ooplasm of many of the young oocytes, situated below the germinal epithelium, was filled with small granules, while in certain parts of the cell, chiefly at one pole of the nucleus, denser clumps of larger granules or rods occurred. The small granules are undoubtedly mitochondria, while the larger ones, owing to their size and disposition, are identified as the Golgi

bodies. The latter, due to the technique employed, are imperfectly preserved.

In young oocytes, with a single follicle layer, masses of darkly stained material occupy similar positions to the Golgi apparatus of the silver and osmic preparations. Mitochondria in the form of fine granules occur distributed through the ooplasm.

In the more advanced oocytes numerous dark granules are scattered through the cell. It was not possible to differentiate with certainty between Golgi bodies and mitochondria; this is due to the clumping of the mitochondria and to the imperfect preservation of the Golgi material. It should be noted that in many of the oocytes fixed in Flemming's fixative no cytoplasmic granules were observed.

In ovaries treated according to Ciaccio's method for the identification of fats, the fats in the corpora lutea and stroma stain brightly with Sudan 111. An examination of the early oocytes revealed bodies in the ooplasm which, although stained with Sudan 111, were not of the same shade as the fats mentioned above. These bodies were of various sizes and shapes, and stained orange-brown. A large number of oocytes at this stage of growth were examined and in all cases the orange-brown bodies occupied similar positions to the Golgi apparatus of the osmic and silver preparations (fig. 4, Pl. 39). Moreover, their subsequent behaviour is similar to that of the Golgi bodies, for, in the young oocytes, they form a mass at one pole of the nucleus and at a later stage become distributed in the ooplasm. In the older oocytes they are more difficult to distinguish than in the earlier cells. There can be no doubt that these are the Golgi bodies which give a reaction with Ciaccio's technique, but do not stain brightly like the fats of the corpora lutea and stroma.

(b) Tubal Eggs.

The oviducts treated by Cajal's technique did not contain many ova, nor were those present as well preserved as the ovarian ova described above. This material, however, showed that the Golgi bodies are smaller in size and are more numerous than in the late ovarian ova, and that the mitochondrial clumps

have increased in number (fig. 10, Pl. 40). An examination of preparations treated by osmic methods confirmed these findings and showed that the Golgi bodies and mitochondria tended to be more numerous towards one pole of the egg. The earliest tubal eggs observed in these preparations had already undergone maturation.

(c) Germinal Epithelium, Follicle-cells, and
Theca-cells.

The Golgi apparatus of the cells of the germinal epithelium consists of a dark mass of material situated at one pole of the nucleus.

The mitochondria occur scattered through the cytoplasm (fig. 2, Pl. 39).

In Cajal preparations a dark juxta-nuclear body is situated at one pole of the follicle-cell nuclei, and is identified as the Golgi apparatus (figs. 5, 6, 7, and 8, Pl. 39). It appears to consist of a very close network, or possibly of rods and granules massed together as in the young oocytes. In the older follicles the Golgi apparatus is similar in appearance (fig. 9, Pl. 39).

The mitochondria, in the form of rods and granules, occur scattered through the cytoplasm, but in the cells of the young follicles are more numerous in the part of the cell adjoining the oocyte (figs. 5, 6, 7, and 8, Pl. 39).

It is worthy of note that the Golgi apparatus in follicles consisting of a single layer of cells is situated in the cytoplasm between the nucleus and the surface of the cell bordering on the oocyte (figs. 5, 6, 7, and 8, Pl. 39); in follicles consisting of several layers of cells the Golgi material, in the majority of cases, is localized at the pole of the nucleus situated towards the oocyte. The mitochondria are evenly distributed throughout the cytoplasm (fig. 9, Pl. 39). An examination of cells, in late follicles, surrounding the follicular cavity revealed the Golgi apparatus of many cells as situated between the nucleus and the surface of the cell directed towards the cavity; in other cases the position of the Golgi material varies from cell to cell. The probable significance of this phenomenon is discussed later (p. 715).

In material treated by Ciaccio's method for the identification of fat, masses of material stain orange-brown with Sudan 111; they occupy similar positions to the Golgi apparatus of the follicle-cells as shown in silver and osmic preparations.

The Golgi apparatus of the theca-cells is in the form of a network or mass of closely applied rods and granules occupying a juxta-nuclear position. The mitochondria are distributed throughout the cell (fig. 1, Pl. 39).

2. The Nucleolus and Nucleolar Extrusions.

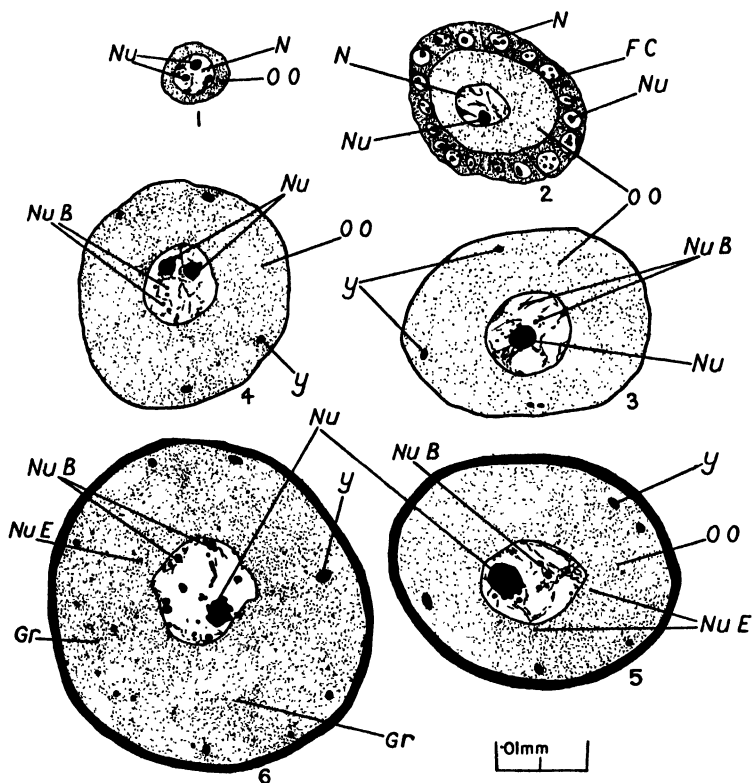
The following investigations on the oocyte nucleolus and nucleolar emissions were carried out chiefly on material fixed in Carnoy, Flemming, and corrosive-acetic fixatives, and subsequently stained in iron-haematoxylin. The basophilia of the nucleoli and nucleolar emissions was determined by preparations fixed in corrosive-acetic and stained in Mann's methyl-blue eosin.

In the early oocytes, situated below the germinal epithelium, one to three nucleoli are usually present; in certain oocytes, however, three to five small nucleoli were observed (Text-fig. 1). In haematoxylin preparations the nucleoli are deeply chromophil, and as revealed by sections stained in Mann's methyl-blue eosin, are basophil.

In young oocytes, with a single layer of follicle-cells, one to three basophil nucleoli are present. In sections stained in iron-haematoxylin the nucleoli are deeply stained homogeneous bodies (Text-fig. 2).

The nuclei of the slightly older oocytes (with about two layers of follicle-cells) contain numerous small homogeneous basophil bodies, many of which are in contact with the single nucleolus, while others are scattered through the nucleoplasm (Text-fig. 3). The position and staining reactions of these bodies point to their nucleolar origin. In certain oocytes a second nucleolus was observed; in most cases the latter contained small vacuoles (Text-fig. 4). Both types of nucleoli and the nucleolar emissions are basophil.

The older oocytes, surrounded by several layers of follicle-cells, usually contain a single deeply-stained nucleolus; in



TEXT-FIGS. 1-6.

LETTERING.

F.C., follicle-cell; *Gr*, granules formed by fragmentation of nucleolar extrusions; *N*, nucleus; *Nu*, nucleolus; *Nu.B*, nucleolar bud; *Nu.E*, nucleolar extrusion; *OO*, ooplasm; *Y*, yolk.

Fig. 1.—Early oocyte situated below germinal epithelium. Carnoy.

Fig. 2.—Early oocyte with single layer of follicle-cells. Carnoy.

Fig. 3.—Oocyte surrounded by two layers of follicle-cells showing nucleolar buds in nucleoplasm and yolk-globules towards periphery of cell. Carnoy.

Fig. 4.—Same stage as fig. 3; two nucleoli are present. Carnoy.

Fig. 5.—Late oocyte; nucleolar extrusions and yolk-globules in ooplasm. Carnoy.

Fig. 6.—Oocyte in mature follicle; numerous granules scattered through ooplasm. Carnoy.

certain oocytes, however, a second vacuolated nucleolus was observed.

The nucleolar emissions are stained deeply by iron-haematoxylin and, as described for the previous stage, are basophil.

The nucleoli appear to be more faintly basophil than in the young oocytes (Text-fig. 5).

In the late oocytes, situated in mature follicles, the nucleolus has lost its spherical shape and, in many cases, appears to be giving rise to numerous nucleolar buds or emissions (Text-fig. 6). In some cases the central part of the nucleolus stained more faintly than the periphery. Both the nucleolus and the nucleolar emissions are basophil. It is of interest to note that a second nucleolus was present in certain of the late oocyte nuclei examined; in these cases both nucleoli stained in the usual manner.

The presence in the older oocytes of nucleolar buds or emissions close to, and in contact with, the inside of the nuclear membrane suggests that these bodies are extruded to the ooplasm. An examination of the late oocytes, surrounded by several layers of follicle-cells, produced further evidence in favour of this view; for numerous small bodies occur on the outside of the nuclear membrane and scattered through the ooplasm in the vicinity of the nucleus and towards the periphery of the cell (Text-figs. 5 and 6). These bodies are closely similar in shape and staining properties to the nucleolar emissions situated in the nucleoplasm; in size they resemble the smaller nucleolar buds. These extrusions were particularly well shown in preparations fixed in Carnoy and in corrosive-acetic.

In no case were nucleolar extrusions observed passing through the nuclear membrane, although in a few oocytes the nuclear membrane seemed to be pushed outward by the presence of emissions on its inner surface. This appearance was probably produced during fixation or the subsequent manipulation of the sections.

Owing to the presence of nucleolar buds in the nucleoplasm and to the occurrence of the same type of structure on both sides of the nuclear membrane, there appears to be little doubt that the bodies situated in the ooplasm are of nucleolar origin. The

nucleolar extrusions are, in all probability, passed through the nuclear membrane in solution and become condensed on reaching the ooplasm.

In some of the late oocytes, in fully-formed follicles, many of the nucleolar extrusions are more lightly stained than those of the younger oocytes, while numerous small faintly-stained granules occur scattered through the ooplasm (Text-fig. 6). The appearance and position of these granules suggest that they may have been formed by the fragmentation of the less deeply-stained nucleolar extrusions. No direct evidence in favour of this view was produced.

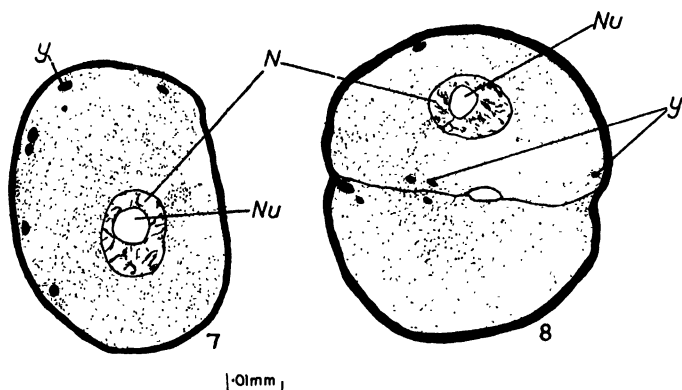
3. Yolk-formation.

In young oocytes, with about two layers of follicle-cells, a number of bodies make their appearance in the ooplasm; they are situated, in the majority of oocytes, towards the periphery but may also occur in the vicinity of the nucleus. These bodies are roughly spherical or somewhat egg-shaped; they stain deeply with iron-haematoxylin except the central portion, which, in many cases, stains but lightly, thus giving to the bodies a vacuolated appearance (Text-figs. 3 and 4).

In order to determine the nature of the cytoplasmic bodies ovaries were treated according to Ciaccio's method for the identification of fat. The fat present in the corpora lutea and in the stroma was deeply stained by Sudan 111, while the cytoplasmic bodies failed to give the correct reaction. Consequently, it was assumed that these bodies were non-fatty. As a further test sections from an ovary fixed in Bouin's piciformol were treated with ether in order to extract any fats which might be present; these sections were subsequently stained in iron-haematoxylin and counter-stained in eosin. An examination of this material showed that the cytoplasmic bodies stained with eosin and were unaffected by the ether. A further proof of the non-fatty nature of these bodies was revealed by the fact that they did not blacken in ovaries fixed by osmic methods. As the result of these tests there can be no doubt as to the non-fatty nature of the cytoplasmic bodies; consequently, they are identified as protein yolk-spheres.

The method of yolk-formation could not be determined with

certainty. In the young oocytes, before the nucleolar extrusions make their appearance in the ooplasm, a few yolk-globules are present; in the late oocytes the yolk-globules are slightly more numerous, and in the majority of cases they have increased in size (Text-figs. 5 and 6). It seems likely that the nucleolar extrusions fragment into granules and that the latter become



TEXT-FIGS. 7 and 8.

Lettering as in figs. 1-6.

Fig. 7.—Unsegmented ovum from upper part of oviduct showing yolk-globules situated towards one pole of the cell. Bouin.

Fig. 8.—Two-cell stage. Bouin.

dissolved in the ooplasm, their substance, in all probability, being added to the yolk-spheres already present. This matter is discussed further on p. 717.

The amount of yolk present in all stages of the growth of the oocyte is scanty; even the late oocytes contain few more yolk-spheres than are present in the young cells (Text-figs. 3, 4, 5, and 6).

An examination of sections of the upper part of oviducts, fixed in Bouin's fixative, showed that the yolk-globules in the unsegmented ova are situated towards one pole of the cell (Text-fig. 7). Yolk-globules were identified in sections of the two-cell stage; they appear to be fairly evenly distributed between the two cells (Text-fig. 8).

4. Atretic Follicles.

As atresia in the mouse has been described by several workers the following account is confined to the description of the cytoplasmic inclusions. For a summary of previous work on atresia see Branca (5).

An examination of oocytes, which, although situated in atretic follicles, had not yet undergone degenerative fragmentation, revealed the majority of the mitochondria (Cajal preparation) as clumped together, forming large masses, while a few occurred scattered through the cell. The Golgi bodies are distributed in small groups or are closely applied to the mitochondrial masses.

Certain of the Flemming material contained a greater number of atretic follicles than were present in the silver preparations; for this reason and also because the corresponding nuclear changes could be followed, thus enabling the stages of degeneration to be determined, the behaviour of the cytoplasmic inclusions was carefully studied in that material. A comparison with the other preparations enabled the Golgi bodies and mitochondria to be identified.

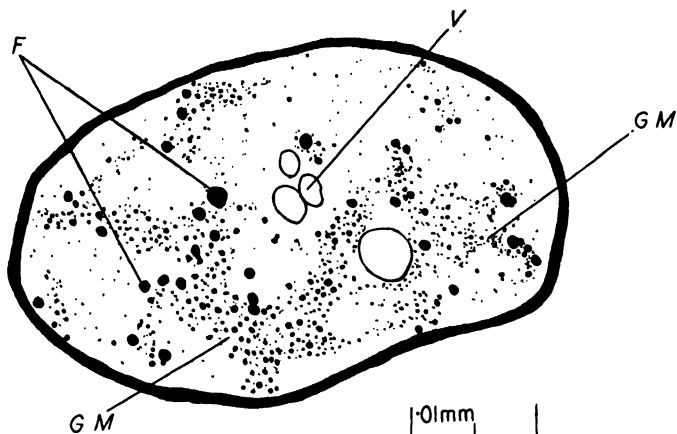
In eggs containing the first spindle the mitochondria occur in clumps and are also scattered through the cell. The Golgi bodies are situated chiefly towards the pole opposite the spindle (fig. 11, Pl. 40).

At a later stage the mitochondrial clumps are larger and denser, and the Golgi bodies are, for the most part, situated towards one pole of the egg (fig. 12, Pl. 40).

In eggs which have fragmented into several pieces the majority of the mitochondria are still clumped and the Golgi bodies distributed unevenly through the cytoplasm. Large vacuoles may be present (fig. 13, Pl. 40).

The number and disposition of the yolk-globules in the early stages of atresia appear to be the same as in the normal oocyte. In the later stages, however, yolk could not be identified. During atresia the cytoplasm becomes filled with numerous fat-globules, and in some cases 'crystalloid bodies' were also observed.

In material treated according to Ciaccio's technique irregularly-shaped granular masses were observed in the cytoplasm; these stain faintly with Sudan 111 and correspond in position to the masses of mitochondria and Golgi elements revealed by silver and osmic methods. It is of interest to note that in most cases fat-globules are more numerous in the vicinity of these granules than in other parts of the cell (Text-fig. 9).



TEXT-FIG. 9.

LETTERING.

F, fat-globules; *G.M.*, Golgi bodies and mitochondria; *V*, vacuole.

Fig. 9.—Oocyte from atretic follicle. Ciaccio.

IV. DISCUSSION.

The above account of the position and behaviour of the juxta-nuclear Golgi apparatus appears to agree fairly closely with the findings of other workers on the Golgi material of the mammalian ovum. The actual structure of the Golgi apparatus of the oocyte has been the subject of a certain amount of disagreement; consequently, before discussing the present findings it is necessary briefly to refer to certain papers on vertebrate oogenesis.

The first observation on the Golgi apparatus in eggs was carried out by Sjövall (26), working on the ovum of *Cavia*,

who states that the Golgi apparatus of the young oocyte is in the form of a hollow sphere situated at one pole of the nucleus. Later, the sphere breaks up and the fragments pass to the periphery. Weigl (Nihoul, **23**) and Kulesch's findings (**20**) agree with those of Sjövall.

According to Rio Hortega (**25**) the Golgi apparatus of the early oocyte of the guinea-pig and rabbit is in the form of a network occupying a juxta-nuclear position. The Golgi elements of later oocytes, in primary follicles, are figured as scattered through the ooplasm in the form of a loose mesh-work or collections of threads united by short extensions. In the older eggs the Golgi material occurs as a loose network of thick threads situated towards the periphery so that a clear space is left surrounding the nucleus.

Gatenby and Woodger (**10**) believe that the mammalian Golgi apparatus 'consists of numerous semi-lunar plates or rods and not of branched straight bodies as drawn by Hortega'; the appearance of branched rods, they state, is possibly due to distortion caused by formalin fixation.

Cattaneo (**6**) states that the Golgi apparatus of the young oocyte of the bat, guinea-pig, and rabbit is in the form of a network situated at one pole of the nucleus. In the older oocytes the apparatus increases in size, breaks up, and passes to the periphery, where it forms a sort of fenestrated membrane in the neighbourhood of the pellucid zone.

Henneguy (**16**), in a short note, states that the Golgi apparatus of the young oocyte of the guinea-pig occurs as 'quelques amas irréguliers de petits cordons granuleux'. They are disposed without order in the ooplasm, but in the late oocyte, surrounded by a follicle with a follicular cavity, they are situated at the periphery.

Nihoul (**23**) believes that the Golgi apparatus of the young oocyte of the rabbit consists of grains or batonnets forming a compact mass at one pole of the nucleus. Later, the Golgi apparatus fragments into several masses which pass to the periphery of the cell. With the growth of the egg it is probable that the substance of the Golgi apparatus increases. In silver preparations of eggs at this stage the Golgi apparatus is con-

stituted 'par une série de travées sans structure, ou présentant une structure finement granuleuse, et anastomosée'. A tangential section of an egg which has just reached this stage gives the impression of a fenestrated membrane similar to that described by Cattaneo. In sections treated according to Weigl's method the Golgi material seems to be formed of filaments which appear as closely entangled and compact masses. The filaments are sometimes curved and give the impression of vesicles of which the wall is strongly coloured and the contents uncoloured. Nihoul believes that the appearance of the Golgi material in silver preparations is due to precipitation on and between the filaments.

The present findings for the mouse agree with those mentioned above in that the Golgi apparatus of the young oocyte is at first in the localized condition and later breaks up and becomes scattered through the ooplasm. In the mouse ovum, however, the Golgi bodies are fairly evenly distributed throughout the cell; furthermore, the individual Golgi elements appear as rods and granules and do not form a loose mesh-work or masses of entangled filaments, as described for the bat, guinea-pig, and rabbit. The present writer believes that the juxta-nuclear Golgi apparatus of the early oocyte is composed of rods and granules which have come together to form a compact mass; this agrees with Nihoul's description of the young oocyte of the rabbit.

It is of interest to note that the localized Golgi apparatus of the early oocytes of certain other vertebrates has recently been described as consisting of collections of individual Golgi elements. Thus Brambell (4) has shown that the Golgi material of the young oocyte of the fowl consists of rods and granules surrounding the centrosphere. Bhattacharya and Lal (2) have figured the Golgi elements in the young oocyte of the tortoise, *Kachuga*, as spherical or granular bodies forming a fairly compact mass beside the nucleus; while more recently Nath (22) states that, in unstained Champy preparations, the Golgi material of the young oocytes of *Rana tigrina* is present as granules occupying a juxta-nuclear position. In Da Fano and Kolatchev material 'they tend to appear as one compact

body'. Later, they increase in size and finally become distributed throughout the ooplasm.

As previously stated (p. 698) Lams and Doorme described two types of mitochondria in the oocyte of the mouse—large granules collected into groups or scattered singly through the cell, and fine granules which in some cases were arranged in short chains. In the opinion of the present writer the large granules of Lams and Doorme are Golgi bodies which, owing to the methods employed, were confused with the mitochondria. It has been shown, in the present paper, that the Golgi elements in the diffuse state tend to collect on the clumps of mitochondria and that imperfectly preserved Golgi bodies are present in certain of the Flemming preparations. It is evident, therefore, that without the aid of osmic and silver methods, suitable for the demonstration of the Golgi material, it would be impossible to distinguish with certainty between the two types of cytoplasmic inclusions.

In an ovum undergoing maturation Lams and Doorme figure the majority of the mitochondria as collected into fairly compact masses; the larger granules appear to be smaller than is the case in the young oocytes. This grouping of the mitochondria agrees with the present findings; furthermore, the Golgi granules and rods of the late ova are slightly smaller than those of the ovarian oocytes and apparently correspond to the large type of mitochondria of Lams and Doorme.

The mitochondria of the early oocyte are more numerous in the neighbourhood of the localized Golgi material; this agrees with Kingery's observation (18) that as the oocyte increases in size the mitochondria come to lie at one end of the cell. The present findings do not agree with Kingery's statement that the mitochondria form a crescent-shaped mass at one end of the cell. According to Kingery the mitochondria later become evenly scattered throughout the cell; the present writer, however, finds that they are less numerous at the periphery, and that in the older oocytes they become collected into clumps.

In view of previous work it is of interest to note that the Golgi material stained faintly with Sudan 111. Bowen (3) has pointed out that, although it is impossible to arrive at any definite

opinion as to the chemical nature of the Golgi apparatus, Ciaccio (7) found that the area of the Golgi apparatus of the testis of the mouse stained with Sudan 111, while Karpova (17), and Parat and Painlevé (24) obtained positive results with the male germ-cells of *Helix*, and Weiner (Bowen, 3), with a modification of Ciaccio's technique, with the cells of the intestinal epithelium of the mouse.

Henneguy (16) states that in the young follicle of the guinea-pig, when it consists of a single layer of cells, the Golgi apparatus is situated between the nucleus and the surface of the cell in contact with the oocyte. In follicles in which the granulosa consists of several layers the situation of the Golgi apparatus varies from cell to cell. In the cells of the *discus proligerus* the Golgi material is situated in the part of the cell directed towards the follicular cavity. Henneguy believes that the follicle-cells which surround the young oocyte secrete a substance which is absorbed by the latter, and that with the appearance of the follicular liquid, which is the product of the cells, an inversion in the situation of the Golgi apparatus takes place.

The position of the Golgi apparatus in the follicle-cells of the mouse, described in the present contribution, is closely similar to that of the Golgi apparatus in the follicle-cells of the guinea-pig previously recorded by Henneguy. In the opinion of the writer the situation of the Golgi material in the young follicle of the mouse strongly suggests that it takes part in the formation of a secretion which is utilized by the growing oocyte. In appearance it closely resembles the polarized Golgi apparatus of gland-cells; the latter, however, is so well known as not to require discussion in this paper. The inversion in the position of many of the Golgi bodies in older follicles would seem to support Henneguy's suggestion that the Golgi material plays some part in the formation of the follicular liquid.

As the mitochondria of the young follicle-cells are more numerous in the part of the cell next to the oocyte, it is reasonable to suppose that the mitochondria, as well as the Golgi apparatus, may take part in the formation of a secretion which is absorbed by the oocyte.

Attention has already been directed to the occurrence, in

degenerating ova, of granules of varying size which take on a faint coloration, and to large fat-globules which stain brightly with Sudan 111 after treatment according to Ciaccio's method for the identification of fats. The masses of smaller granules correspond in position to the clumps of mitochondria and Golgi elements of osmic and silver preparations. The larger granules are apparently Golgi bodies which stain more deeply with Sudan 111 than do those of the normal oocyte. Their staining reaction, together with the occurrence of granules intermediate in size between the smaller granules and the fat-globules, suggests that the Golgi elements, in degenerating eggs, give rise to fat.

Bell (1) has recently described the origin of neutral fats from the Golgi bodies of the spermatid of the dog, while the origin of fatty yolk from the Golgi element of the oocyte has been recorded by several workers. Reference to recent work on fatty yolk-formation has been made in previous contributions by the present writer (11 and 12).

As many of the granules are extremely small and as the surrounding cytoplasm appears to stain faintly with Sudan 111, thus forming a faintly granular background to the larger granules, it is possible that the mitochondria, as well as the Golgi elements, may give rise to fat-globules.

The number of nucleoli observed during the present investigation agrees with Kingery's findings (18); the occurrence of nucleolar extrusions, however, does not appear to have been previously recorded for the mouse ovum. It is of interest to note that although Lams and Doorme (21) do not mention nucleolar emissions they figure what seem to be nucleolar buds in an oocyte nucleus.

The occurrence of nucleolar emissions on the inside of the nuclear membrane, and of closely similar bodies outside the nuclear membrane and scattered through the ooplasm, offers strong evidence in favour of the view that nucleolar material is extruded from the nucleus. It is highly probable that this material is passed through the nuclear membrane in solution and is condensed into granular form on reaching the ooplasm. This view of nucleolar extrusion has been put forward by Harvey for Carcinus (13) and for Antedon (15).

The presence of small granules in the ooplasm suggests that the nucleolar extrusions finally fragment in a somewhat similar manner to that recently described by the writer (12) for *Periplaneta*.

The yolk-globules present in all stages of the mouse oocyte are not numerous; they appear chiefly towards the periphery of the oocyte, but a few may be situated in the neighbourhood of the nucleus. They were not observed to arise in relation to the cytoplasmic inclusions.

Harvey (13 and 14) has recently stated his belief that protein yolk arises under the influence of the mitochondria and Golgi bodies from material derived from the plasmosome, ground cytoplasm, and from external sources. If this be true it is probable that the mitochondria, Golgi elements, or both, play some part in the formation of the scanty yolk of the mouse ovum. Material derived from the nucleolar extrusions may be added to the yolk-globules, as suggested by Harvey (13) for the protein yolk of *Carcinus*. Owing to the large number of nucleolar extrusions present, and to the scanty amount of yolk, it is probable that much of the nucleolar material remains dissolved in the ooplasm, possibly in the form of nutriment which is utilized at a later stage.

Lams and Doorme (21) state that fat-globules are present at the periphery of the mouse oocyte and that they are faintly brown-black after osmic acid and appear as clear spaces after corrosive-acetic fixation. The present investigation shows that these globules, as demonstrated by Ciaccio's method, by the action of ether, acetic and osmic acid, are non-fatty in nature. The only ooplasmic fat-globules present are those of the oocytes situated in atretic follicles. These fats stain (Ciaccio's method) in a similar manner to those of the corpora lutea and stroma cells; the latter have recently been described by Deanesly (8 and 9).

Fat-globules in degenerating eggs of the mouse have previously been recorded by Kingery (19) and by Branca (5). The 'crystalloid bodies' described by Kingery were observed during the present investigation.

V. SUMMARY.

1. The Golgi apparatus of the germinal epithelium consists of a dark mass of material situated at one pole of the nucleus. The mitochondria occur scattered throughout the cytoplasm.

2. The Golgi material of the very early oocyte consists of rods and granules clumped together to form a large body at one pole of the nucleus; smaller masses of Golgi material may also be present.

3. In the young oocyte, surrounded by a follicle wall, a single juxta-nuclear body is present; at a later stage the individual Golgi elements break away from the juxta-nuclear body and become distributed throughout the ooplasm.

4. In the late oocytes the Golgi elements occur in close association with the mitochondrial clumps and also scattered through the ooplasm. In tubal eggs the Golgi bodies are smaller in size and more numerous than in the ovarian ova.

5. It is concluded that the large mitochondria of Lams and Doorme correspond to the oocyte Golgi elements of the present contribution. The behaviour of the Golgi material during the growth of the ovum resembles that of the eggs of other mammals. The present findings on the structure of the juxta-nuclear Golgi material agrees with Nihoul's account for the rabbit.

6. The mitochondria of the young oocytes occur scattered through the ooplasm, but are more numerous in the vicinity of the nucleus and Golgi material. Later, the majority of the mitochondria become collected into clumps; in the tubal eggs the mitochondrial clumps are more numerous.

7. The Golgi apparatus of young follicles is situated between the follicle-cell nucleus and the pole of the cell directed towards the oocyte; in follicles consisting of several layers the position of the Golgi apparatus varies, while in fully-formed follicles the Golgi material of many of the cells surrounding the follicular cavity are directed towards the cavity. This agrees with Henneguy's findings for the Golgi apparatus of the follicle-cells of the guinea-pig. The mitochondria of the follicle-cells occur scattered through the cytoplasm but are more numerous towards the pole of the cell adjoining the oocyte.

8. The number of nucleoli present in the early oocyte varies from one to five; the majority of the older oocytes contain a single nucleolus but two may be present. Extrusion into the ooplasm of nucleolar material takes place; the nucleoli and the nucleolar extrusions are basophil (Mann's methyl-blue eosin).

9. Fatty yolk is not present in the mouse ovum. It is suggested that the Golgi elements and mitochondria play some part in yolk-formation, and that some of the granules formed by the fragmentation of the nucleolar extrusions are added to the yolk-globules already present. The yolk-globules of unsegmented tubal eggs are situated towards one pole of the cell; at the two-cell stage they appear to be evenly distributed between the two cells.

10. In degenerating eggs the mitochondria are clumped; the Golgi bodies occur in small groups or are closely applied to the mitochondrial clumps. In eggs which have undergone fragmentation the Golgi bodies occur in groups, while the majority of the mitochondria are clumped. The fat-globules, previously recorded by Kingery in degenerating eggs, were identified. In material treated by Ciaccio's method for the identification of fats, appearances suggest that the Golgi elements, and possibly the mitochondria, give rise to fat. Yolk-globules could not be distinguished in the late stages of these eggs.

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EXPLANATION OF PLATES 39 AND 40.

LETTERING.

F.C., follicle-cell; *G.*, localized Golgi material; *G.E.*, Golgi element; *M.*, mitochondria; *M.C.*, mitochondrial clump; *N.*, nucleus; *Nu.*, nucleolus; *V.*, vacuole.

PLATE 39.

Fig. 1.—Theca-cells showing Golgi apparatus and mitochondria. Cajal.

Fig. 2.—Germinal epithelium showing Golgi apparatus and mitochondria. Cajal.

Fig. 3.—Early oocyte; follicle wall not formed. Cajal.

Fig. 4.—Oocyte at same stage as fig. 3. Ciaccio.

Fig. 5.—Oocyte with single layer of follicle-cells. Cajal.

Fig. 6.—Slightly later stage showing Golgi elements breaking away from juxta-nuclear clump. Cajal.

Fig. 7.—Later stage; most of the Golgi elements have become distributed throughout the ooplasm. Cajal.

Fig. 8.—Older oocyte with single layer of follicle-cells; the majority of the mitochondria are collected into clumps; the Golgi elements are distributed throughout the ooplasm. Cajal.

Fig. 9.—Late oocyte; Golgi bodies and mitochondria clumped in ooplasm; the position of the Golgi apparatus of the follicle-cells varies from cell to cell. Cajal.

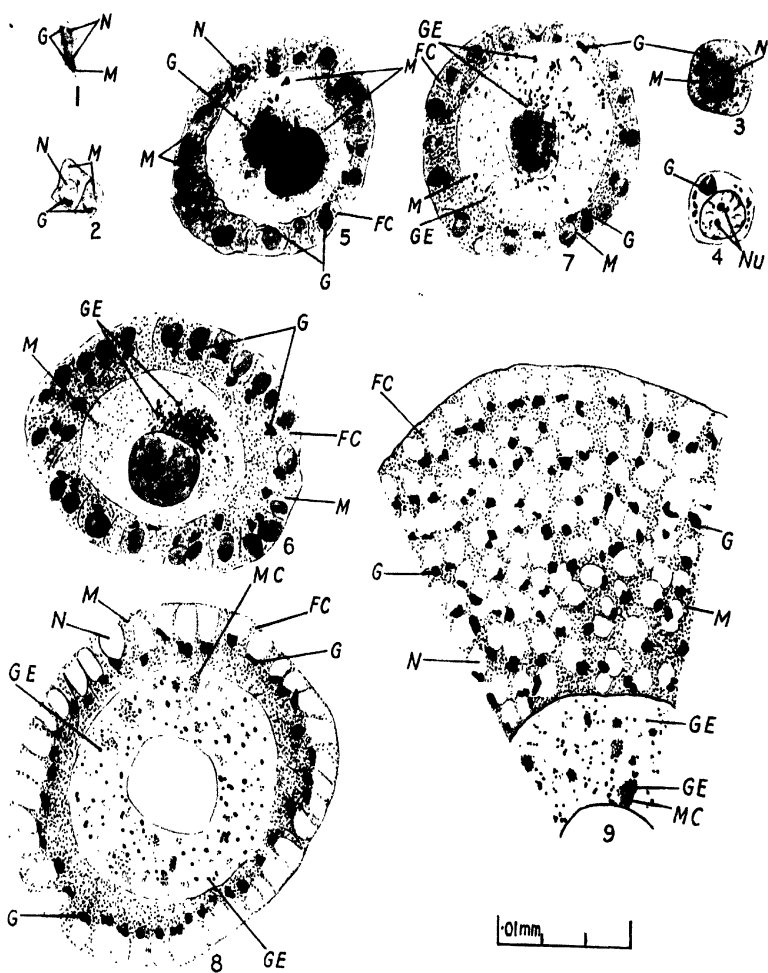
PLATE 40.

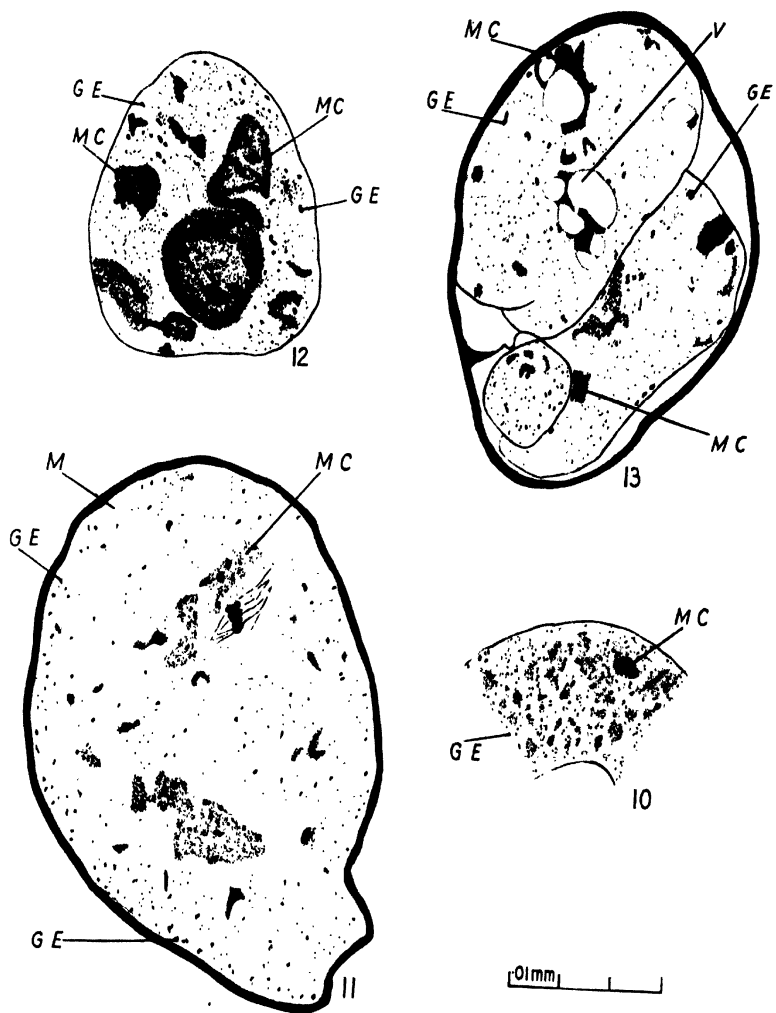
Fig. 10.—Part of ovum from upper part of oviduct. Cajal.

Fig. 11.—Oocyte from atretic follicle showing arrangement of Golgi elements and mitochondria. Flemming.

Fig. 12.—Later stage of degeneration. Cajal.

Fig. 13.—Egg which has fragmented into several pieces. Flemming.





The Nephridia of *Asymmetron* and *Branchiostoma* compared.

By

Edwin S. Goodrich, F.R.S.

With 12 Text-figures.

THE excretory organs of *Branchiostoma* (= *Amphioxus*) are so peculiar, in that they resemble the protonephridia of many invertebrates and differ radically from the excretory tubules of other vertebrates (Goodrich, 1902 and 1909), that it is a matter of some importance to ascertain whether organs of similar structure occur in the allied genus *Asymmetron*. Andrews, so far as I am aware the only author who has dealt with this genus in detail, failed to find them in the *Asymmetron lucayanum* he described (Andrews, 1893, p. 229). During a recent visit to Bermuda I obtained specimens of this species,¹ and give here the results of the study of fresh and preserved material carried out in the Bermuda Biological Station during last July and August and since in Oxford. A comparison is also made with the excretory organs of *Amphioxus*.

Before embarking on the description of the nephridia of *Asymmetron* something must be said about those of *Branchiostoma*, since, in spite of all that has been written about them, there are still some points in their structure which seem to be misunderstood.

Branchiostoma lanceolatum Pallas (= *Amphioxus lanceolatus* Yarrell).

The Paired Nephridia.—The size and complexity of the nephridium in full-grown individuals does not seem to be generally appreciated. Excellent as are the well-known figures of Boveri (1892) in many respects (notwithstanding the

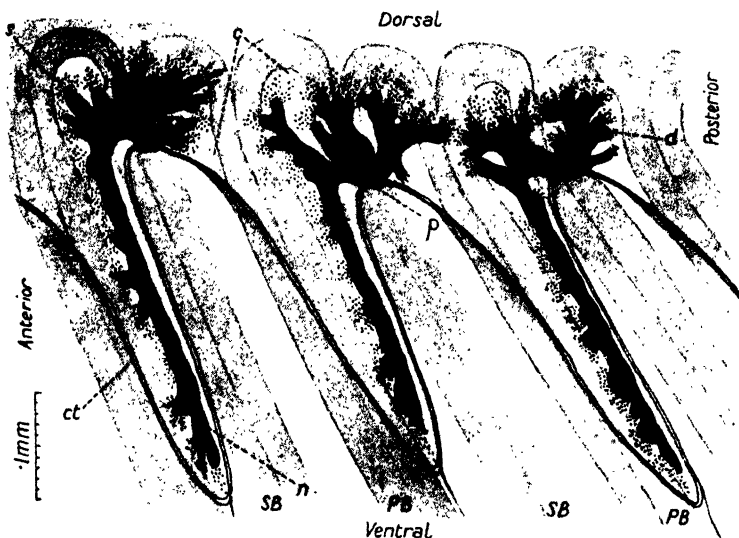
¹ I am greatly indebted to Dr. J. F. G. Wheeler, Director of the Bermuda Biological Station, and to Prof. E. G. Conklin for helping me to collect this elusive animal.

erroneous introduction of open coelomic funnels, and the misrepresentation of the solenocytes) they give but little idea of the size and branching of a well-developed nephridial canal. Nor do the figures of Franz (1926, 1927) provide a better picture. My own figure of the whole nephridium (1902) is more adequate; but, since the gill-bars and other associated parts are not drawn, the real proportions of the organ are not obvious to the reader.

To supply this deficiency a new figure is here given (Text-fig. 1), drawn to scale with camera lucida from a whole mount of the wall of the pharynx, showing three consecutive nephridia in position on the gill-bars. On this scale the solenocytes appear very small; they were not drawn with the camera, and were no doubt more numerous than indicated in the figure. It will be noticed that the number of blind branches is great, and that the length of many of them is considerable. More particularly can it be seen that the main anterior (rostral) canal, running down the cavity of the ligamentum denticulatum attached to the primary bar, may be over 0.3 mm. long.

A second and more important point to be mentioned concerns the relation of the tubes of the solenocytes to the wall of the nephridial canal recently discussed by Dr. V. Franz. Although Franz agrees that, "Öffnungen ins Cölon haben die Nephridien zweifellos nicht" (1926, p. 548), he asserts that the tubes of the solenocytes do not pierce the wall of the nephridium as described by me (and also by Legros in the nephridium of Hatschek, 1910), either in the paired nephridia or in Hatschek's nephridium. He states that, 'Ich bin dagegen überzeugt, das der von einen Solenocytenbündel durchbohrte Bereich an plasmatischen Massen nur die Solenocytenstiche enthält, die also wirklich als ein Bündel aufgefasst werden müssen und das Loch im Nephridium vollständig ausfüllen' (1927, p. 569), and gives two figures to support his view. There is no evidence that he has critically examined this point on living material. Neither in the living, nor in whole preparations, nor in the hundreds of sections I have examined, have I ever seen the appearance depicted in Franz's Text-figs. 46 and 57. This point was dealt with in great detail in a previous paper (Goodrich,

1909), and I still believe my description is correct. Numerous figures were given in that paper proving, I think quite conclusively, that the basal end of the solenocyte tube is actually embedded in the cytoplasmic wall of the canal, though in places



TEXT-FIG. 1.

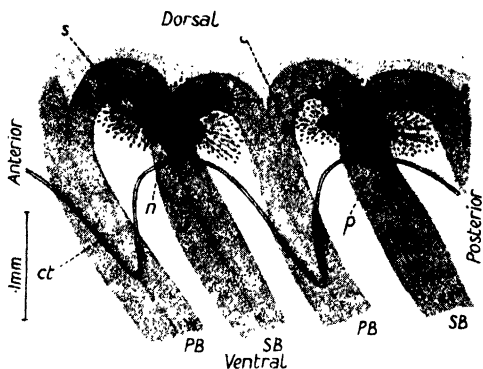
Portion of dorsal region of wall of pharynx of *Branchiostoma lanceolatum*, drawn with camera lucida from a stained preparation. Cut edges of denticulate ligament drawn diagrammatically.

LETTERING FOR TEXT-FIGURES 1-12.

ao, lateral aorta; *at*, atrial epithelium; *br*, blood-vessel; *c*, lateral dorsal or suprapharyngeal coelom; *cep*, coelomic epithelium; *co*, ciliated wheel organ; *ct*, cut edge of atrial wall or denticulate ligament; *d*, diverticulum of nephridial canal; *dsc*, dorsal solenocyte chamber; *ep*, ectodermal epithelium of roof of oral-hood cavity; *ibr*, inner branch or diverticulum of Hatschek's nephridium; *l*, lumen of nephridium; *lc*, longitudinal canal of Hatschek's nephridium; *n*, nephridium; *nat*, nucleus of atrial epithelium; *nc*, nephridial canal; *ncep*, nucleus of coelomic epithelium; *np*, nephridiopore; *ns*, nucleus of solenocyte; *nt*, notochord; *nts*, sheath of notochord; *PB*, primary gill-bar; *s*, solenocyte; *SB*, secondary gill-bar; *sc*, medial or inner ventral solenocyte chamber; *t*, tube of solenocyte; *tbr*, blind tip of branch of Hatschek's nephridium.

where the tubes are very numerous very little cytoplasm separates them from each other. The absence of nuclei in the wall of this region of the canal is explained on the assumption that the solenocytes themselves are derived from it, and, so to speak, drawn out from the wall in connexion with which they remain by means of the lengthening tubes (Goodrich, 1902 and 1909).

In one case only do I readily admit that the adverse criticism of Franz is justified. There is an unfortunate 'slip of the pen'



TEXT-FIG. 2.

Portion of dorsal region of wall of pharynx of *Asymmetron lucayanum*, drawn with camera from a stained preparation. Cut edges of denticulate ligament drawn diagrammatically.

on p. 187 of my paper (1909), where the external opening of the nephridium is said to occur opposite the 'anterior' instead of the posterior (caudal) edge of the secondary gill-bar. Franz is right in saying that the nephridiopore is posterior to the attachment of the secondary bar, though it appears about the middle of the bar in Text-fig. 1 owing to slight distortion caused by the pressure of the cover-glass.

Hatschek's Nephridium.—Some years ago I gave an account of the structure of the unpaired nephridium of Hatschek in the adult *Branchiostoma lanceolatum* (1909).

The reader may be reminded that it is a true protonephridium similar in general structure to the posterior paired nephridia.

It extends along the outer side of the left aorta in the head region, reaching from just in front of Hatschek's pit backwards to the pharynx into which it opens dorsally behind the velum. Very numerous solenocytes are set mostly on the short blind diverticula of its main longitudinal canal. It has no internal opening, and lies 'morphologically' in a narrow cavity closed in the adult, but in communication with the mysclerocoel of the second segment in the larva, and clearly derived therefrom. The canal runs along the ventro-lateral wall of this cavity which is practically obliterated in the adult leaving, however, small chambers at intervals. It is into these chambers lying on the ventral and lateral sides of the aorta that the bunches of solenocytes on the diverticula project.

The above description is taken from my previous paper (1909). It has been adversely criticized by Dr. Franz in two publications (1926 and 1927).

He says 'das H. N. [Hatschek's nephridium] liegt nicht, wie frñhen vermutet wurde, in einer Cölomhñhle, sondern ist ins Gallertbindegewebe eingebettet, in welchem nur die Solenocyten von einem eigenen, an die Wand der linken Aorta (Karotide) rñhrenden Hohlraum umschlossen sind' (1926, p. 554). The same statement is repeated in much the same words in 1927 (p. 539). Now, although it is true that in the adult the cavity enclosing this nephridium is rather virtual than real, it nevertheless persists as a cavity in the form of the chambers into which project the solenocytes along the course of the nephridial tube. As described and figured in my previous paper (1909), the cavity in the larva is continuous with the scleromyocoel of segment 2 until a late stage when the right series of gill-slits is about to develop. According to my observations the closing off of the nephridial cavity is brought about by the downgrowth of a connective tissue septum on the inner or medial side of the ventral end of the first left myomere, which septum meeting the ventral wall of the myocoel eventually cuts off the latter space from the nephridial cavity lying next to the aorta (Goodrich, 1909, Pl. 14, figs. 25 and 26). Legros (1910) also finds that the cavity into which extend the solenocytes of Hatschek's nephridium is derived from the middle

region (vésicule intermédiaire) of the coelomic cavity of the second segment and later gives rise to the 'chambres à soléno-cytes'. If I understand him correctly, his description of the way in which the cavity becomes cut off agrees with my own.

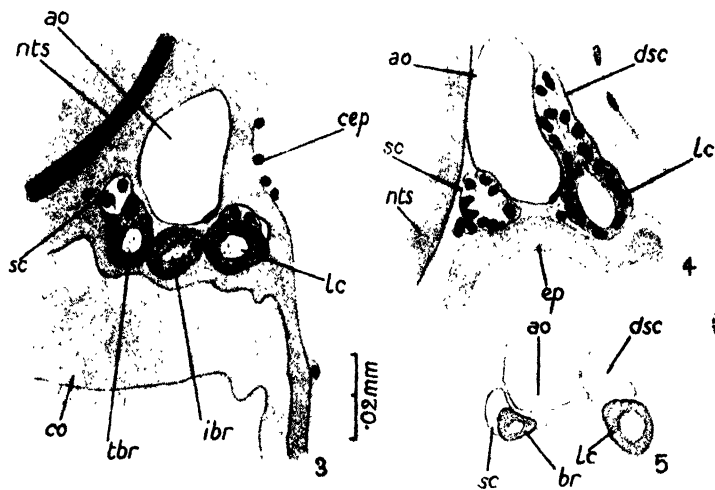
In larval stages, when the cavity is still in open communication with the scleromyocoele, it is easy to trace the coelomic epithelium into the nephridial chamber (Pl. 14, fig. 26, 1909); but later on, as already mentioned, the cavity is practically obliterated round the main nephridial canal, though small spaces here and there in addition to the chambers may be remains of it. In the adult the coelomic epithelium is very indistinct and usually no longer visible even in the solenocyte chambers. Certain nuclei, however, may be seen occasionally on the wall of the chambers or on the main canal which doubtless represent remnants of the coelomic epithelium.¹

Franz further maintains that "Das H. N. hat nicht zahlreiche Kurze Verzweigungen oder 'Divertikel' wie nach Boveri und Goodrich die Kiemennephridien, sondern ist—gegen Goodrich—eine unverzweigte—nur in einem Ausnahmefalle sich einmal gabelnde—Röhre" (1926, p. 554).

Now, although it is possible that in my figure (1909, Pl. 14, fig. 27) the length of some of these diverticula may have been slightly exaggerated for the sake of clearness, there can be no possible doubt that they exist in the full-grown adult. In order to make this quite clear some new figures are here given (Text-figs. 3, 4, 5). There are in fact two series of such diverticula or branches from the main longitudinal canal: an inner ventral series passing towards the middle line below the left aorta, and an outer dorsal series passing upwards laterally to the aorta. Each diverticulum has a blind end provided with a bunch of solenocytes projecting into a chamber. The chambers are well-shown in Text-fig. 4. A ventral branch in Text-fig. 3 is shown to be some 0.035 mm. long. In Text-figs. 3 and 5 the lumen of a branch appears cut separately from the lumen of the longitudinal canal in the same transverse section. Indeed,

¹ The development of Hatschek's nephridium will be dealt with in a later paper.

Text-fig. 3 shows a third lumen, since the diverticulum has a somewhat bifurcated extremity.



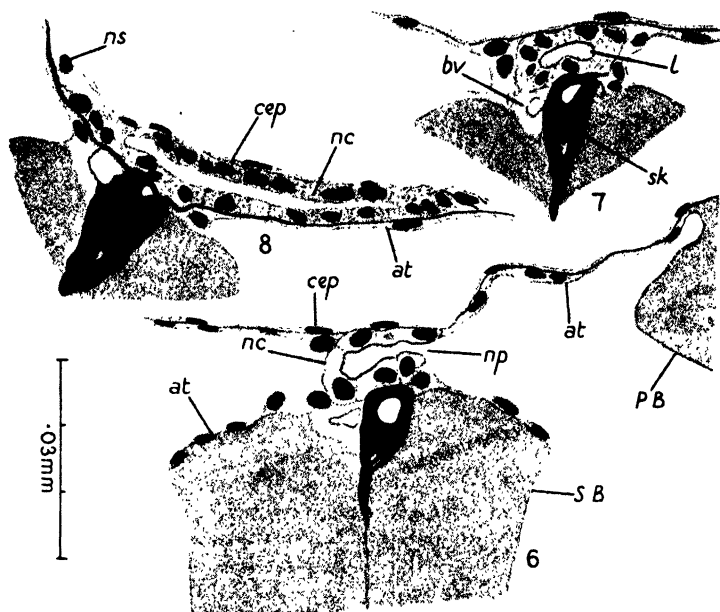
TEXT-FIGS. 3, 4, and 5.

Transverse sections of Hatschek's nephridium of *Branchiostoma lanceolatum*, drawn with camera lucida at same magnification.

Asymmetron lucayanum Andrews.

The Paired Nephridia.—The nephridia of *Asymmetron* are built on the same plan as those of *Branchiostoma*, but they are smaller and simpler. Here also one nephridium corresponds on each side to one primary gill-slit throughout the length of the pharynx. Except in the case of the first slit, which as in *Branchiostoma* remains undivided by a tongue bar, the external pore opens into the atrium near the dorsal end of the secondary bar (Text-figs. 2 and 6). From the pore the canal runs dorsally and expands into a somewhat triangular sac with anterior and posterior corners sometimes considerably recurved ventrally. Even the anterior limb, which is generally the longer, never reaches the primary bar. The solenocytes spring mostly from near the dorsal edge of the

nephridial sac and also from its outer surface. They are numerous and spread over a solenocyte-field on the inner wall of the longitudinal suprapharyngeal coelomic cavity. The whole nephridium is morphologically 'retroperitoneal', lying mostly



TEXT-FIGS. 6, 7, and 8.

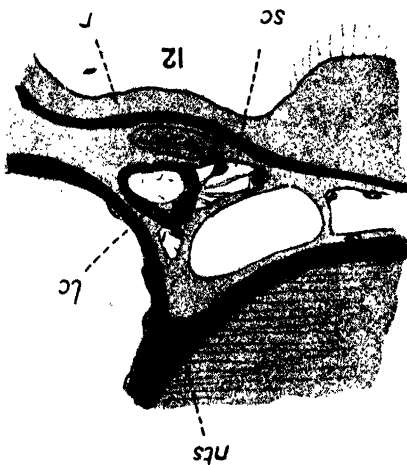
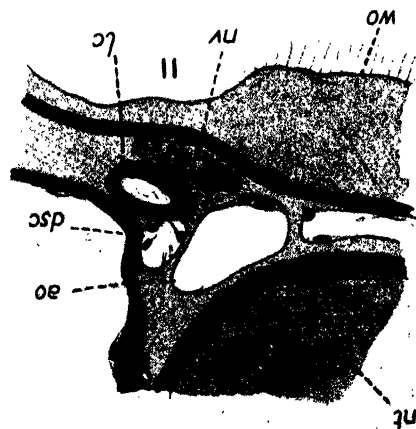
Sections of nephridium of *Asymmetron lucayanum* cut at right angles to gill-bar.

Fig. 6.—Most ventral and through external pore.

Fig. 8.—Most dorsal. Camera lucida.

between the coelomic epithelium and the atrial epithelium; but, while the nephridial canal or sac is covered on its outer side with a distinct layer of coelomic epithelium, this epithelium is interrupted over the solenocyte-field so that the solenocytes and their tubes are bathed by the coelomic fluid (Text-figs. 6, 7, and 8) as in *Branchiostoma* (Goodrich, 1909).

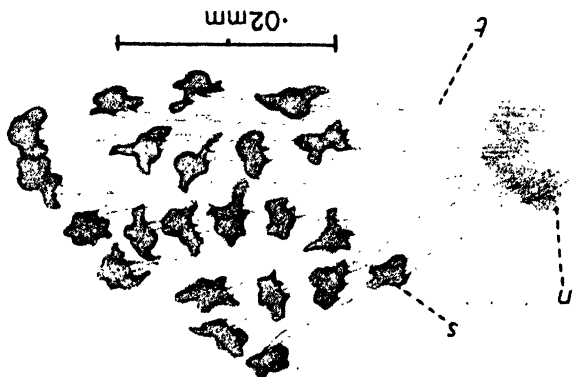
The solenocytes themselves are rather larger than in *Branchiostoma*. The cell-bodies containing the nucleus appear



TEXT-FIGS. 11 AND 12.

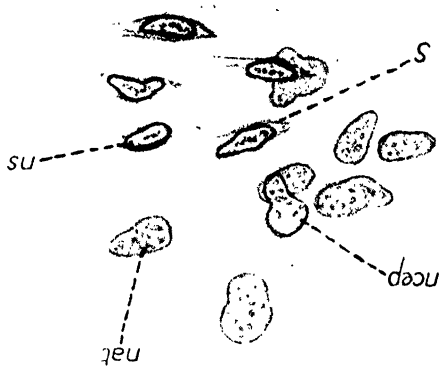
Asymmetron lucayanum. Transverse sections of Hatschek's nephridium taken at level of Hatschek's pit; camera lucida.

solenocyte, down which works a long flagellum, may reach a length of about 0.04 mm., and seems less rigid than in Bran-



TEXT-FIG. 9.

Asymmetron lucayanum. Portion of solenocyte-field showing distribution of solenocytes. Drawn from living; camera lucida.



TEXT-FIG. 10.

Asymmetron lucayanum. Edge of solenocyte-field from stained preparation; camera lucida.

more irregular in shape with usually many delicate processes of the cytoplasm. A thick process anchors the cell firmly to the wall of the field over which solenocytes are fairly regularly distributed; but two cell-bodies may often be seen united by a bridge of cytoplasm (Text-figs. 9 and 10). The tube of the

